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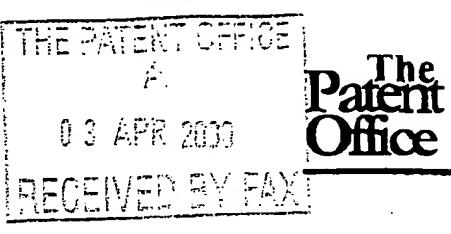
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	Patents ADP number (if you know ii)	United Kingdom	7367100	000
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١.	Title of the invention	COMPOUNDS FOR TARGET	ring	
5.	Name of your agent (if you have one)	ERIC POTTER CLARKSON	V	
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COMPOUNDS FOR TARGETING

The present invention relates to cytotoxic compounds that have a high avidity for, and can be targeted to, selected cells. Specifically, the invention provides compounds comprising a cytotoxic portion having DNA endonucleolytic activity and a target-cell specific portion having specificity for human polymorphic epithelial mucin (PEM).

10 Background

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The cell-specific targeting of compounds that are directly, or indirectly, cytotoxic has been proposed as a way to combat diseases such as cancer. Bagshawe and his co-workers have disclosed (Bagshawe (1987) Br. J. 15 Cancer 56, 531; Bagshawe et al (1988) Br. J. Cancer 58, 700; WO 88/07378) conjugated compounds comprising an antibody or part thereof and an enzyme, the antibody being specific to tumour cell antigens and the enzyme acting to convert an innocuous pro-drug into a cytotoxic compound. The cytotoxic compounds were alkylating agents, e.g. a 20 benzoic acid mustard released from para-N-bis(2chloroethyl)aminobenzoyl glutamic acid by the action of Pseudomonas sp. CPG2 enzyme.

An alternative system using different pro-drugs has been disclosed (WO 91/11201) by Epenetos and co-workers. The cytotoxic compounds were cyanogenic monosaccharides or disaccharides, such as the plant compound amygdalin, which release cyanide upon the action of a β-glucosidase and hydroxynitrile lyase.

In a further alternative system, the use of antibody-enzyme conjugates containing the enzyme alkaline phosphatase in conjunction with the prodrug etoposide 4'-phosphate or 7-(2'-aminoethyl phosphate)mitomycin or a combination thereof have been disclosed (EP 0 302 473; Senter et al (1988) Proc. Natl. Acad. Sci. USA 85, 4842).

Rybak and co-workers have disclosed (Rybak et al (1991) J. Biol. Chem. 266, 21202; WO 91/16069) the cytotoxic potential of a monomeric pancreatic ribonuclease when injected directly into Xenopus oocytes and the cytotoxic potential of monomeric RNase coupled to human transferrin or antibodies directed against the transferrin receptor. The monomeric RNase hybrid proteins were cytotoxic to human erythroleukaemia cells in vitro.

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Other approaches are the *in vivo* application of streptavidin conjugated antibodies followed, after an appropriate period, by radioactive biotin (Hnatowich *et al* (1988) *J. Nucl. Med.* 29, 1428-1434), or injection of a biotinylated mAb followed by radioactive streptavidin (Paganelli *et al* (1990) *Int. J. Cancer* 45, 1184-1189). A pilot radioimmunolocalisation study in non-small cell lung carcinomas was conducted with encouraging results (Kalofonos *et al* (1990) *J. Nucl. Med.* 31, 1791-1796).

Apart from these examples, it is rather more common to see biotinylated antibodies and streptavidin-enzyme conjugates, which are used in enzyme-linked immunosorbent assays.

These previous systems have used relatively large antibody-enzyme,

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antibody-streptavidin or antibody-biotin conjugates and may comprise portions of non-mammalian origin which are highly immunoreactive.

We have now devised improved compounds for targeting cells to be destroyed.

Summary of Invention

A first aspect of the invention provides a compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity.

By "target cell specific" portion we mean the portion of the compound which comprises one or more binding sites which recognise and bind to polymorphic epithelial mucin (PEM) on the target cell. Upon contact with the target cell, the target cell specific portion is preferably internalised along with the cytotoxic portion. Such internalisation results in the cytotoxic portion being delivered to the cell cytosol, where it has access to the cell's nucleic acid molecules.

The target cell-specific portion of the compounds of the invention comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof.

Polymorphic epithelial mucin, or PEM, is a component of the human milk

fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

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Monoclonal antibodies which will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigenspecific portion may be a whole antibody, a part of an antibody (for example a Fab or F(ab')₂ fragment), a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]), or a peptide/peptidomimetic or similar. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", J G R Hurrell (CRC Press, 1982) and Antibody Engineering, A Practical Approach, McCafferty, J. et al, ed. (IRL Pres, 1996).

By 'humanised monoclonal antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complimentarity-determining region (CDR) is derived from a second, donor monoclonal antibody having specificity for PEM. The donor monoclonal antibody may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably, both chains of the humanised monoclonal antibody comprise

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CDRs grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (i.e. humanised) chain comprises two or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human framework residues and CDRs from a donor antibody having specificity for PEM.

However, it will be appreciated by those skilled in the art that in order to maintain and optimise the specificity of the humanised antibody it may be necessary to alter one or more residues in the framework regions such that they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are derived from an human IgG monoclonal antibody.

- Methods of making humanised monoclonal antibodies are well-known in the art, for example see Jones et al. (1986) Nature 321:522-525, Riechmann et al. (1988) Nature 332:323-327, Verhoeyen et al. (1988) Science 239:1534-1536 and EP 239 400 (to Winter).
- In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises an humanised HMFG-1 monoclonal antibody or an antigen binding fragment thereof.

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimiriou et al., 1981, Int. J. Cancer 28:17-21 and Gendler et al., 1988, J. Biol. Chem. 236:1282-12823). HMFG-1 monoclonal antibodies bind to a particular component of HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the amino acid sequence APDTR within the twenty amino acid tandem repeats of the muc-1 gene product.

Examplary humanised HMFG-1 antibodies are disclosed in WO 92/04380.

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Advantageously, the target cell-specific portion is an humanised HMFG-1 monoclonal antibody.

In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises a fragment of an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), said fragment retaining the antigen binding properties of the parent antibody.

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison et al (1984) Proc. Natl. Acad. Sci. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving

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the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); disulphide-linked Fv molecules (Young et al., 1995, FEBS Lett. 377:135-139); single-chain Fv (ScFv) molecules where the V_R and V_L partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293-299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are linked via a flexible oligopeptide.

Chimaeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. Fab, Fv, ScFv, disulphide Fv and dAb antibody fragments can all be expressed in and secreted from bacteria, such as E. coli, or eukaryotic expression systems such as Yeast or mammalian systems, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F(ab')₂ fragments are "bivalent". By "bivalent" we

mean that the said antibodies and F(ab')₂ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, disulphide Fv and dAb fragments are monovalent, having only one antigen combining site.

Preferably, the target cell-specific portion of the compounds of the invention comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')₂, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

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More preferably, the target cell-specific portion comprises a Fab molecule or a F(ab')₂ molecule.

Yet more preferably, the target cell-specific portion comprises at least a part of one or both of the amino acid sequences shown in Figure 3.

Most preferably, the target cell-specific portion comprises both of the amino acid sequences shown in Figure 3.

20 Preferably, the target cell-specific portion recognises the target cell with high avidity.

By "high avidity" we mean that the target cell-specific portion recognises the target cell with a binding constant of at least $K_d = 10^{-6} \,\mathrm{M}$, preferably at least $K_d = 10^{-9} \,\mathrm{M}$, suitably $K_d = 10^{-10} \,\mathrm{M}$, more suitably $K_d = 10^{-11} \,\mathrm{M}$, yet more suitably still $K_d = 10^{-12} \,\mathrm{M}$, and more preferably $K_d = 10^{-15} \,\mathrm{M}$ or even $K_d = 10^{-18} \,\mathrm{M}$.

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Preferably, the target cell-specific portion comprises an antigen binding fragment of an humanised HMFG-1 monoclonal antibody, e.g. an Fab or $F(ab')_2$ fragment thereof, wherein a hinge region contains a mutation (i.e. wherein the hinge is a variant or hybrid of a naturally occurring hinge). More preferably, the variant hinge comprises the amino acid sequence CCVECPPCPAPE.

By 'cytotoxic portion' we mean a portion having endonucleolytic activity which is toxic to the cell if it is to reach, and preferably enter said cell.

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In a preferred embodiment of the first aspect of the invention, the cytotoxic portion has DNA endonucleolytic activity.

Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

Examples of known DNA endonucleases include bovine DNase I (see Worrall and Conolly, 1990, J. Biol. Chem. 265:21889-21895). Human pancreatic DNase I has also been cloned (see Shak et al., 1990, Proc. Natl. Acad. Sci. USA 87:9188-9192 and Hubbard et al., 1992, New Eng. J. Med. 326:812-815).

Preferably, the endonuclease is a mammalian deoxyribonuclease I.

25 More preferably, the endonuclease is a human deoxyribonuclease I.

Most preferably, the cytotoxic portion comprises the amino acid sequence shown in Figure 2.

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Preferably, the cytotoxic portion of the compound of the invention is capable of oligomerisation, e.g. dimerisation. Attachment of the target-cell specific portion to a cytotoxic portion capable of oligomerisation provides a method for increasing the number of binding sites to the target cell. For example, if the target cell-specific portion is joined to a portion capable of forming a dimer then the number of target cell-specific binding sites is two; if the target cell-specific portion is joined to a portion capable of forming a tetramer then the number of target cell-specific binding sites is four. The number of target cell-specific binding sites is greater than one and the compounds may therefore have a greater avidity for the target cell than do compounds which only have one target cell-specific binding site.

It is preferable for the cytotoxic portion of the compound of the invention capable of oligomerisation to contain no interchain disulphide bonds nor intrachain disulphide bonds; to be well characterised; to be non-toxic; to be stable; to be amenable to preparation in a form suitable for pre-clinical or clinical use or be in pre-clinical or clinical use; and for the subunit monomers to have a high affinity for each other, that is they contain one or more subunit binding sites.

Advantageously, the cytotoxic portion is of mammalian, preferably human, origin. The use of the said mammalian proteins as the cytotoxic portion of the compound of the invention is advantageous since such compounds are less likely to give rise to undesirable immune reactions.

It will be appreciated by those skilled in the art that the cytotoxic portion

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may be a variant of a naturally occurring endonuclease.

By "a variant" we include cytotoxic portions comprising of a naturally occurring endonuclease wherein there have been amino acid insertions, deletions or substitutions, either conservative or non-conservative, such that the changes do not substantially reduce the endonuclease activity of the variant compared to that of the naturally occurring endonuclease. For example, the variant may have increased activity compared to the naturally occurring endonuclease

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Such variants may be made using methods of protein engineering and site-directed mutagenesis commonly known in the art (for example, see Sambrook et al., 1989, Molecular cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, NY, USA).

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In an alternative embodiment, the endonuclease is a restriction endonuclease, such as a microbial type II restriction endonuclease. Exemplary type II restriction endonucleases include *BamHI*, *HindIII*, *MspI*, *Sau3AI*, *HinfI*, *NotI* and *EcoRI*.

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In another preferred embodiment of the first aspect of the invention, a nuclear localization signal is incorporated into the compound.

Preferably, the nuclear localization signal comprises a nuclear localization signal from the SV40 large T antigen (Kalderon et al., 1984, Cell 39:499-509), and specifically the amino acid sequence PKKKRKV. Inclusion of a nuclear localization signal encourages the compound of the invention to gain access to the chromosomal DNA during the periods of the cell sycle

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when the nuclear membrane is intact, since the nuclear pores are permeable to large molecules incorporating said nuclear localization signal.

In a further preferred embodiment of the first aspect of the invention, the target cell-specific portion and the cytotoxic portion are fused to create a fusion compound.

By "fusion compound" we include a compound comprising one or more functionally distinct portions, wherein the distinct portions are contained within a single polypeptide chain produced by recombinant DNA techniques.

Preferably, the target-cell specific and the cytotoxic portion of the fusion compound of the invention separated by a linker sequence, for example to allow greater flexibility of the portions relative to one another.

More preferably, the linker sequence comprises a GG dipeptide.

20 Most preferably the linker sequence is or comprises GG or GSGG.

Alternatively, the target-cell specific and the cytotoxic portion of the compound of the invention are separate moieties linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan et al Anal. Biochem. (1979) 100, 100-108. For example, the antibody portion may be enriched with thiol groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide

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ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

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A second aspect of the invention provides a nucleic acid encoding a compound according to the first aspect of the invention, or a target cell-specific portion or cytotoxic portion thereof.

By "nucleic acid molecule" we include DNA, cDNA and mRNA molecules.

A further aspect of the present invention provides a method of making a compound according to the first aspect of the invention, said method comprising expressing one or more nucleic acid molecules according to the second aspect of the invention in a host cell and isolating the compound therefrom.

It is preferable that the two portions of the compound of the invention are produced as a fusion compound by recombinant DNA techniques, whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. The benefits in making the compound of the invention using recombinant DNA techniques are several fold. Firstly, it enables a high degree of precision with which the two portions of the compound can be joined together. Secondly, the construction of compounds which are "hetero-oligomeric" can be

controlled by the expression of the different recombinant DNA molecules encoding each of the different type of subunit of the "hetero-oligomer" in the same host cell.

- By "hetero-oligomer" we mean those compounds in which two or more different cell-specific portions are joined to either the same or to different subunits which are capable of oligomerisation. The expression, in the same host cell of two compounds, of A and B, each with different target cell specific portions but with a common second portion capable of oligomerisation will result in a mixed population of compounds. For example, if the common second portion is capable of dimerisation, three potential compounds will be produced: A₂, AB and B₂, in a ratio of 1:2:1, respectively.
- The separation of the desired compound with each of the different cell specific portions, that is AB, can be achieved by two step affinity chromatography.
- Application of the mixture of compounds to an affinity column specific for 20 A will result in the binding of A₂ and AB. These compounds are eluted from this first column, and then applied to an affinity column specific for B. This will result in AB, but not A₂, being bound to the column. Finally, the desired product AB, can be eluted.
- Of course, the order in which the affinity columns are used is not important.

The same principle of separating those compounds with two or more

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different binding sites can be applied to the purification of the desired compounds from mixtures of other hetero-oligomers.

Conceivably, the two portions of the compound may overlap wholly or partly.

The nucleic acid is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the nucleic acid encoding the compound of the invention or a portion thereof may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter et al., 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark et al., 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura et al., 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. et al., 4,766,075 issued 23 August 1988 to Goeddel et al and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The nucleic acid encoding the compound of the invention or a portion thereof may be joined to a wide variety of other nucleic acid sequences for introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or integration is desired.

It will be appreciated that in order to prevent expression of the cytotoxic portion of the compound of the invention from killing the host cells in which it is expressed, it may be necessary to link the nucleic acid of the second aspect of the invention to a signal sequence capable of directing secretion of the expressed compound (or portion) out of the host cell. Signal sequences will be selected according to the type of host cell used. Exemplary signal sequences include the *ompA* signal sequence (for example, see Takahara *et al.*.1985, *J. Biol. Chem.* 260(5):2670-2674).

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Generally, the nucleic acid is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a nucleic acid sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

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Host cells that have been transformed by the recombinant nucleic acid of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings

disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells (for example COS-1, COS-7, CHO, NIH 3T3, NS0 and BHK cells) and insect cells (for example Drosophila, SF9 cells).

- Those vectors that include a replicon such as a procaryotic replicon can also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.
- A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

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Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 (available from Biorad Laboratories, Richmond, CA, USA), pTrc99A and pKK223-3 (available from Pharmacia Piscataway, NJ, USA) and the pET system (T7 promoter, Novagen Ltd).

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A typical mammalian cell vector plasmid is pSVL available from Pharmacia. Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of

expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers his3, trp1, leu2 and ura3. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Further useful vectors for transformation of yeast cells, such as *Pichia*, include the 2μ plasmid pYX243 (available from R and D Systems Limited) and the integrating vector pPICZ series (available from Invitrogen).

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

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Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described

earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

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The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

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Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to modify the nucleic acid encoding the compound of the invention or a portion thereof is to use the polymerase chain reaction as disclosed by Saiki et al (1988) Science 239, 487-491.

In this method the nucleic acid to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified nucleic acid. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

Exemplary genera of yeast contemplated to be useful in the practice of the present invention are Pichia, Saccharomyces, Kluyveromyces, Candida, Torulopsis, Hansenula, Schizosaccharomyces, Citeromyces, Pachysolen, Debaromyces, Metschunikowia, Rhodosporidium, Leucosporidium, Botryoascus, Sporidiobolus, Endomycopsis, and the like. Preferred genera are those selected from the group consisting of Pichia, Saccharomyces, Kluyveromyces, Yarrowia and Hansenula. Examples of Saccharomyces are Saccharomyces cerevisiae, Saccharomyces italicus and Saccharomyces rouxii. Examples of Kluyveromyces are Kluyveromyces fragilis and Kluyveromyces lactis. Examples of Hansenula are Hansenula polymorpha, Hansenula anomala and Hansenula capsulata. Yarrowia lipolytica is an example of a suitable Yarrowia species.

Methods for the transformation of S. cerevisiae are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

Suitable promoters for S. cerevisiae include those associated with the PGK1 gene, GAL1 or GAL10 genes, CYC1, PHO5, TRP1, ADH1, ADH2, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, α-mating factor pheromone, a-mating factor pheromone, the PRB1 promoter, the GUT2 promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (eg the promoter of EP-A-258 067).

The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, i.e. may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the S. cerevisiae AHD1 gene is preferred.

The present invention also relates to a host cell transformed with a 10 polynucleotide vector construct of the present invention. The host cell can be either procaryotic or eukaryotic. Bacterial cells are preferred procaryotic host cells and typically are a strain of E. coli such as, for example, the E. coli strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No 15 ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the 20 ATCC as CRL 1658 and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 or WSØ cells.

Transformation of appropriate cell hosts with a nucleic acid constructs of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen et al. *Proc. Natl. Acad. Sci. USA*, 69: 2110 (1972); and Sambrook et al,

Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989). Transformation of yeast cells is described in Sherman et al, Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor, NY (1986). The method of Beggs. Nature. 275: 104-109 (1978) is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc, Gaithersburg, MD 20877, USA.

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Successfully transformed cells, i.e. cells that contain a nucleic acid construct of the present invention. can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern. J. Mol. Biol., 98: 503 (1975) or Berent et al, Biotech., 3: 208 (1985). Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

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In addition to directly assaying for the presence of recombinant nucleic acid, successful transformation can be confirmed by well known immunological methods when the recombinant nucleic acid is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains the protein.

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

A third aspect of the invention provides a vector comprising a nucleic acid according to the second aspect of the invention.

A fourth aspect of the invention provides a host cell comprising a vector according to the third aspect of the invention.

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Preferably, the host cell is a mammalian cell.

More preferably the host cell is NSO.

A fifth aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

The compounds and compositions of the invention are administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or, preferably (for bladder cancer), intravesically (i.e. into the bladder), in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered

intravenously).

A sixth aspect of the invention provides a compound according to the first aspect of the invention for use in medicine.

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The compounds and compositions of the invention may be used to treat a patient with any disease involving a dysfunction of a population of cells expressing PEM, said compounds and compositions selectively targeting and destroying said population of cells within a patient. For example, said compounds and compositions may be used in the treatment of cancer, e.g. cancer of the breast, ovaries, lung, stomach, intestines, blood etc. Thus, anti-tumour cell antigen antibodies can be used to deliver a cytotoxic portion with endonuclease activity to a tumour cell. Antibodies that are internalised upon contact with the target antigen are used, such that the cytotoxic portion enters the cytosol of the tumour cell, where it can trigger cell death.

In principle, the compounds and compositions of the invention may be used to treat any mammal, including pets such as dogs and cats and agriculturally important animals such as cows, horses, sheep and pigs.

Preferably, the patient is human.

A seventh aspect of the invention provides the use of a compound according to first aspect of the invention in the preparation of a medicament for treating a mammal having said target cells to be destroyed.

Preferably, the medicament is for treating cancer, such as ovarian cancer.

A eighth aspect of the invention provides a method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to the first aspect of the invention to said mammal.

In a preferred embodiment of the seventh and eighth aspects of the invention, the mammal is a human.

- Preferably, the target cells to be destroyed are cancer cells. More preferably, the cancer cells are epithelial cancer cells, such as ovarian, gastric, colorectal and/or pancreatic cancer cells. Most preferably, the cancer cells are ovarian cancer cells.
- The invention will now be described in detail with reference to the following figures and examples:

Figure 1 shows the complete coding sequence of human DNAse I.

Figure 2 shows the DNAse I sequence used in the exemplary constructs (e.g. pAS41).

Figure 3 shows the HMFG1 light chain insert (A) and heavy chain insert (B) used in the exemplary constructs (e.g. pAS6).

Figure 4 shows the linker and hinge-linker oligonucleotides used in (A) the whole antibody-DNase and (B) the Fab'2-DNase exemplary constructs. Note, in Figure 4(A) a deletion of one or more codons

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between the HMFG1 hinge and the linker is represented as $\triangle G$.

Figure 5 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS23 (linker sequence underlined).

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Figure 6 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS27 (linker sequence and NLS underlined).

Figure 7 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS34 (linker sequence underlined).

Figure 8 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS35 (linker sequence underlined).

The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 9 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS36 (linker sequence underlined).

The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 10 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS37 (linker sequence and NLS underlined).

Figure 11 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS38 (linker sequence and NLS

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underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 12 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS39 (linker sequence underlined).

The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 13 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS101 (linker sequence underlined).

Figure 14 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS102 (hybrid hinge and linker sequence underlined).

Figure 15 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS103 (hybrid hinge and linker sequence underlined).

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Figure 16 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS104 (hybrid hinge and linker sequence underlined, mutations (compared to pAS103) at positions 775 and 924 are shaded).

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Figure 17 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS105 (linker sequence and NLS underlined).

Figure 18 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS106 (hybrid hinge + linker sequence and NLS are underlined).

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Figure 19 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of exemplary construct pAS107 (hybrid hinge + linker sequence and NLS are underlined).

Figure 20 shows a schematic diagram of the pEE6 expression vector used in the exemplary constructs.

Figure 21 shows autoradiographs from immuno-precipitation experiments with metabolically labelled transient transfectants:

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GEL A

Lane 1 shows the precipitation of supernatant from mock-transfected cells.

Lane 2 is from cells transfected with hHMFG-1 (construct 6) giving expected molecular weights of about 51.2 and 26.4 kDa for the heavy and light chains, respectively.

Lane 3 shows construct 34 antibody construct which has human DNase I fused to the C-terminus of the heavy chain gene. As expected, the size of the heavy chain gene has increased to about 80.7 kDa

25 80.7 kDa.

Samples from whole antibody DNase I constructs 35, 36 and 39 were run on the gel (Lanes 4 to 6) but were not sufficiently well expressed to be visible, in this experiment.

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In subsequent experiments using this method, construct 39 was detectable but weak, and constructs 35 and 36 were detectable but very weak. Constructs 37 and 38 have not been tested in this assay system.

Lanes 8 to 10 are fusion of humanised HMFG1 F(ab')₂ with human DNase I (constructs 41, 23 and 102, respectively). F(ab')₂ alone was included in this set of experiments (lane 7, construct 41) but did not express, this was included in later experiments (see gels C and D). In addition to the light chain (about 26.4 kDa) and the Fd-DNAse I fusion (about 56.6 kDa), a third major band is observed at around 40 kDa. Interestingly, this band is observed in the humanised HMFG-1 fusions but not in the antibody alone. Since an anti-F(ab')₂ antibody was used for immuno-precipitation, it is unlikely that this can be proteolysis between immunoglobulin and DNase I sequence. It probably represents a population of polypeptide produced by premature transcriptional termination (due to DNase I sequence in the 3'-end of the fusion mRNA).

GEL B

This is the non-reducing gel counterpart to gel A, described above.

Lane 1 is the mock-transfected control cells and lanes 2 and 3 are from the cells transfected with humanised HMFG1 alone (construct 6) and the humanised HMFG-1 fused at the C-terminus to human DNase I, respectively. As before, lanes 4 to 6 are from cell supernatants from cells transfected with constructs 35, 36 and 39.

The gel shows that both the whole antibody and the antibody-DNase I fusion are assembled, with the DNase fusion giving a higher molecular weight compared to the antibody alone.

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Figure 22 shows a typical standard curve used to determine the concentration of PDTRP-binding material in the supernatants of transiently transfected L761h cells. Each point on the curve has been determined twice.

- Figure 23 shows typical standard curves used to determine the concentration of bovine DNAse I.
- Figure 24 shows corrected DNase I activity in transiently expressed humanised HMFG1 whole antibody-human DNAse I fusions (i.e. pAS34, pAS35 and pAS6[control]).
- Figure 25 shows the corrected DNAse I activity in transiently expressed humanised HMFG1 F(ab')₂-human DNase I fusions (i.e. pAS101, pAS102, pAS103 and pAS41[control]).
 - Figure 26 shows results of the cytotoxicity assay.
- Figure 27 shows the % of MCF7 cells killed afetr incubation with the exemplary constructs.

EXAMPLES

(A) Mammalian expression of humanised HMFG1-DNase constructs

5 The human HMFG1 light and heavy chain (with or without engineering a fusion to human DNase I), were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NSO cells (see figure 20). The vector system was originally developed by Celltech Ltd (UK) and is now owned by al-Lonza (see Young & Owens, 1994, J. Immunol. Meth. 168:149-165). The vector consists of two human 10 cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. Propagation in E. coli can be selected for by the presence on an ampicillin resistance 15 gene (not shown in Fig 20). The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by growth in glutamine free media, since NSO cells are GSO and cannot otherwise grow in glutamine free media.

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Exemplary humanized HMFG1-DNAse I fusion constructs of the invention are detailed in figures 5 to 19.

(B) <u>Immuno-precipitation of metabolically labelled transient</u> 25 <u>transfectants</u>

CHO-L761h cells (Cockett et al., 1990, Nuc. Acids Res. 19:319-325) were transfected, according to the modification of Gorman et al, 1985),

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with expression vectors containing either whole HMFG1 antibody or $F(ab')_2$ fragment of the antibody along with the various fusion constructs of their respective heavy chains and human DNase I. The cells were then incubated with either 50 μ Ci ³⁵S methione for 72 h in methionine-free medium. Secreted product was precipitated with a rabbit anti-human $F(ab')_2$ antibody bound to protein A Sepharose. Bound material was eluted in either reducing or non-reducing SDS-PAGE loading buffer and run on gels. The autoradiographs (see Figure 21) above were generated from those gels after drying them.

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(C) Estimation of the efficiency of DNase constructs in supernatants

Introduction

This set of experiments was designed to standardise the amount of construct in a given DNase I activity assay and to allow us to comment on the amount of activity a particular construct possesses. Given that the antibody-DNase I fusions are so different to the F(ab')₂-DNase I fusions it is best not to compare the two groups. Once we have purified the protein, we will have a better idea of the exact molecular configuration of all species. Then, and only then, will it be sensible to compare amongst groups.

Determination of concentration of constructs

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The concentration of constructs in supernatants from transiently transfected L761H cells was determined in a PDTRP-binding ELISA. To each well of a Maxisorb 96-well ELISA plate (Nunc) was added 100 μ l of

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carbonate buffer containing 100 ng of recombinant GST-(PDTRP)₇ fusion protein (Gendler et al., 1990, J. Mol. Biol. 265:15286-93). After overnight binding at 4°C, the plate was washed three times in PBS-Tween (i.e. PBS containing 0.05% Tween-20). The plate was then blocked with three 3-minute washes of PBS-Tween containing 1% BSA.

For each construct, $100 \mu l$ of supernatant was added to a well on the plate. In addition, hHMFG-1 of known concentration was serially diluted down the plate using doubling dilutions in $100 \mu l$ of PBS-Tween per well. The plate was incubated for a further 1 h at 30° C, then 200 ng of MC135 antihuman kappa light chain antibody (binding site) in $100 \mu l$ of PBS-Tween was added to each well for 1 h at 30° C. After three 3-minute washes in PBS-Tween, $100 \mu l$ of anti-mouse IgG-peroxidase conjugate (Jackson 315-035-045), diluted 1:2000 in PBS-Tween, was added to each well and incubated for 1 h at 30° C. Following a final set of three 3-minute washes in PBS-Tween, $100 \mu l$ of TMB substrate (Sigma) was added to each well of the plate and, after a colour developed, the optical density at 630 nm of the solution in each well of the plate was determined.

20 Results.

(see Figure 22)

(D) Corrected bovine DNase I standard curves and DNase assay

DNase activity was determined using a modification of the methyl green-DNA complex degradation method (Sinicropi et al., 1994, Analyt. Biochem. 222:351-358). Briefly, a 1:1 solution of the assay buffer and methyl green-salmon sperm DNA complex was mixed together to give a total volume of 0.2 ml. To this, 0.1 ml of tissue culture supernatant from transiently transfected CHO-L761h cells was added and the mixture incubated at 37°C. DNA cleavage by DNase results in a reduction in absorbance at 620 nm. Figure 23 shows a standard curve produced with various concentrations of bovine DNase I over a number a time point.

Figures 24 and 25 show DNAse activity for the whole HMFG1 antibodyand $F(ab')_2$ - DNase fusions, respectively.

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(E) Cytotoxicity of DNAse constructs

Method

DNase constructs were transfected into CHO L761h cells using a calcium phosphate co-precipitation method (Gorman et al., 1985, In: DNA cloning (2nd edition), Glover A(ed.), Academic Press, NY, 163-188). Included in the experiment were negative controls, consisting of cells transfected with TE buffer alone or with TE buffer and pEE6 expression vector. In addition to these controls, vectors that express hHMFG-1 (pAS6) and F(ab')₂ of hHMFG1 (both with specificity for PEM but without DNase I) were included.

The supernatant from these cells was harvested after 72 h of expression, followed by centrifugation to remove dead cells. MCF-7 cells were incubated for 1 h at 37°C with an aliquot of each of these supernatants. The amount of cellular lactate dehydrogenase (LDH) released from the MCF-7 cells due to the cytotoxicity of the supernatant was determined

using the CytoTox96 cytotoxic assay kit (Promega). Total lysis ('total LDH') was determined by measuring the target cell maximum LDH release using the kits lysis solution. The percentage of cells killed was then calculated as the proportion of the LDH released to the total LDH released. For each construct, the cytotoxicity assay was performed in quadruplicate, except for assay of pAS38 and 39, which were performed in triplicate. The values of LDH release for each construct were compared against either $F(ab')_2$ or whole antibody, or each other, using a one-tailed t-test in Excel.

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Results

Figures 26 and 27 shows that there is negligible cell killing with either pAS6 (HMFG1 alone) or with pAS41 ($F(ab')_2$ alone). All of the hHMFG1 $F(ab')_2$ -DNase I constructs kill significantly more cells than the $F(ab')_2$ fragment alone (p<0.00193) and all of the antibody-DNase I constructs kill significantly more cells than antibody alone (p<0.00783), except for perhaps pAS34 (p<0.021).

20 (F) Use of the DNase-I/huHMFG-1 Fab fusion protein in the treatment of ovarian cancer

Patients diagnosed with ovarian cancer are treated by intravenous injection of the DNaseI/huHMFG-1 Fab fusion protein. Typically, a dose of between 1 to 100 mg will be administered weekly.

Therapeutic response is measured by the normal clinical procedures that are well known in the art, for example radio-imaging methods.

CLAIMS

- 1. A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that:
 - (i) the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof; and
 - (ii) the cytotoxic portion has endonucleolytic activity.
- 2. A compound according to Claim 1 wherein the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof.
- 3. A compound according to Claim 2 wherein the target cell-specific portion is an humanised HMFG-1 antibody.
- 4. A compound according to Claim 1 or 2 wherein the target cell-specific portion comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')₂, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).
 - 5. A compound according to Claim 4 wherein the target cell-specific portion comprises a Fab molecule.
 - 6. A compound according to Claim 4 wherein the target cell-specific

portion comprises a F(ab')₂ molecule.

- 7. A compound according to Claim 1 wherein the target cell-specific portion comprises at last part of one or both of the amino acid sequences of Figure 3.
- 8. A compound according to Claim 7 wherein the target cell-specific portion comprises both of the amino acid sequences of Figure 3.
- 9. A compound according to any one of Claims 1 to 8 wherein the cytotoxic portion has DNA endonucleolytic activity.
- 10. A compound according to Claim 9 wherein the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.
- 11. A compound according to Claim 10 wherein the endonuclease is a mammalian deoxyribonuclease I.
- 12. A compound according to Claim 11 wherein the endonuclease is a human deoxyribonuclease I.
- 13. A compound according to Claim 1 wherein the endonuclease is a restriction endonuclease.
- 14. A compound according to Claim 10 wherein the cytotoxic portion comprises the amino acid sequence of Figure 2.
- 15. A compound according to any one of Claims 1 to 14 wherein a nuclear

localization signal is incorporated.

- 16. A compound according to Claim 15 wherein the nuclear localization signal comprises the sequence PKKKRKV.
- 17. A compound according to any one of Claims 1 to 16 wherein the target cell-specific portion and the cytotoxic portion are fused.
- A compound according to Claim 17 wherein the target cell-specific portion and the cytotoxic portion are separated by a lniker sequence.
- 19. A compound according to Claim 18 wherein the linker sequence is or comprises GG or GSGG.
- 20. A nucleic acid encoding a compound as defined in any one of Claims 17 to 19.
- 21. A vector comprising a nucleic acid according to Claim 20.
- 22. A host cell comprising a vector according to Claim 21.
- 23. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 19 and a pharmaceutically acceptable carrier.
- 24. A compound according to any one of Claims 1 to 19 for use in medicine.
- 25. Use of a compound according to any one of Claims 1 to 19 in the

preparation of a medicament for treating a mammal having said target cells to be destroyed.

- 26. A method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to any one of Claims 1 to 19 to said mammal.
- 27. A use according to Claim 25 or a method according to Claim 26 wherein the mammal is a human.
- 27. A use according to Claim 25 or a method according to Claim 26 wherein the target cells to be destroyed are cancer cells.
- 28. A use or a method according to Claim 27 wherein the cancer cells are epithelial cancer cells.
- 29. A use or a method according to Claim 28 wherein the cancer cells are ovarian, gastric, colorectal and/or pancreatic cancer cells.
- 30. A use or a method according to Claim 29 wherein the cancer cells are are ovarian cancer cells.
- 31. A compound substantially as described herein, preferably with reference to one or more of the accompanying figures.

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ABSTRACT

Compounds for Targeting

A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity. Preferably, the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof. Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease, e.g. a human DNA endonuclease I. The invention further provides nucleic acids encoding the compounds of the invention, and the use of such compounds in medicine, e.g. in the treatment of cancer.

FIGURE 1

Human DNase I

```
LOCUS
                HUMDNASEI
                             1039 bp
    DEFINITION
                                        mRNA
                Human DNase I mRNA, complete cds.
                                                        PRI
                                                                   06-MAR-1995
    ACCESSION
    VERSION
                M55983.1 GI:181623
    KEYWORDS
                DNase I.
    SOURCE
                Human pancreus, cDNA to mRNA.
     ORGANISM
               Homo sapiens
               Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
               Eutheria; Primates; Catarrhini; Hominidae; Homo.
   REFERENCE
     AUTHORS
               Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
     TITLE
               Recombinant human DNase I reduces the viscosity of cystic fibrosis
     JOURNAL
               Proc. Natl. Acad. Sci. U.S.A. 97 (23), 9188-9192 (1990)
    MEDLINE
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                      LFVYRPDQVSAVDSYYYDDGCEPCGNDTFNREPAIVRFFSRFTEVREFAIVPLHAAPG
                      DAVAEIDALYDVYLDVQEKWGLEDVMLMGDFNAGCSYVRPSQWSSIRLWTSPTFQWLI
                      PDSADTTATPTHCAYDRIVVAGMLLRGAVVPDSALPFNFQAAYGLSDQLAQAISDHYP
     gene
                      160..1008
                      /gene="DNase I"
     mat peptide
                     226..1005
                     /gene="DNase I"
                     /product="DNase I"
BASE COUNT
                226 a
                         305 ¢
ORIGIN
                                  282 g
                                           226 t
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     121 cattctcgtc atctctgagg acatcaccat catctcagga tgaggggcat gaagctgctg
     181 ggggcgctgc tggcactggc ggccctactg cagggggccg tgtccctgaa gatcgcagcc
     241 ttcaacatcc agacatttgg ggagaccaag atgtccaatg ccaccctcgt cagctacatt
     301 gtgcagatce tgagccgcta tgacatcgcc ctggtccagg aggtcagaga cagccacctg
     361 actgccgtgg ggaagctgct ggacaacctc aatcaggatg caccagacac ctatcactac
     421 gtggtcagtg agccactggg acggaacagc tataaggagc gctacctgtt cgtgtacagg
     481 cctgaccagg tgtctgcggt ggacagctac tactacgatg atggctgcga gccotgcggg
    541 aacgacacct tcaaccgaga gccagccatt gtcaggttct tctcccggtt cacagaggte
    601 agggagtttg ccattgttcc cctgcatgcg gccccggggg acgcagtagc cgagatcgac
    661 gctctctatg acgtctacct ggatgtccaa gagaaatggg gcttggagga cgtcatgttg
```

721 atgggcgact tcaatgcggg ctgcagctat gtgagaccct cccagtggtc atccatccgc

781 ctgtggacaa gececaeett ecagtggetg ateceegaca gegetgacae cacagetaca

841 cccacgcact gtgcctatga caggatcgtg gttgcaggga tgctgctccg aggcgccgtt 901 gttcccgact cggctcttcc ctttaacttc caggctgcct atggcctgag tgaccaactg

11

961 gcccaagcca teagtgacca ctatecagtg gaggtgatge tgaagtgage agecceteee 1021 cacaccagtt gaactgeag

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MIDNASE. DI

g181623

753 bp

IRS

Ruman DNace I mRNA, complete cds, Mature sequence modified to remove Narl site

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ficure 2

LOCUS

NID

Definition ACCESSION

human VN/se I ansmit

MPNA

```
TEINOXD2
             DNase I.
 SOURCE
             Buman pancrous, CDNA to mRNA.
  ORGANISM
             Homo sapiens
             Eukaryotae; mitochondrial cukaryotes; Metazoa; Chordata;
             Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
 RÉFERENCE
                (bases 1 to 1039)
            Shak, S., Capon, D.J., Hellming, R., Marsters, S.A. and Baker, C.L.
  ACTHORS
   TITLE
            Recombinant human DNage I reduces the viscosity of cystic fibrosis
  JOURNAL
            Proc. Natl. Read. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE
             91067672
PEATURES
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                     EDVMLMCDFNACCSYURPSQNSSIRLWTSPTFQWLIPDSADTTATPTHCAYDRIVVAG
                     MILLRGAVVPDSALE:FNFQAAYGLSDQLAQAISDHYPVEVMIK"
     Gene
                     160. .7008
                     /douca_pwage i.
     mat_peptide
                     226. .1005
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                     /product=*Dwage I*
BASE COUNT
                168 a
                         236 c
                                  550 d
                                           159 t
ORIGIN
       I CIGAAGAICG CAGCCTICAA CAICCAGACA TTTGGGGAGA CCAAGAIGTC CAATGCCACC
      61 CICGTCAGCI ACATIGTGCA GAICCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC
     121 AGRGACAGOC ACCIGACTGO CETGGGGBAG CTGCTGGACA ACCTCAATCA GGACGCACCA
     181 GACACCIATO ACIACGIGOT CAGIGAGCCA CIGGGACGGA ACAGUIAIAA GGAGCGCIAC
     241 CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC
     301 TECHACCOT GOGGANOGN CACCTTORAC CGAGAGOCAG COATIGTORG GITCITCICC
     361 CEGTICAÇAG AGCICAGGA GITIGCCATI GITCCCCIGO AICCCCCCC CGGGGACGCA
     421 CTACCCCACA TEGACGETEI CTATGACGIC TACCIGGAIG ICCAAGAGAA AIGGGGCIIG
     481 GAGGACGICA IGIIGAIGEG CGACTICAAT GCGGGCTGCA GCTAIGTGAG ACCCICCCAG
     541 TOGTCATCCA TEEGECTGTG GACAAGECEE ACCITECAGT GGETGATEEE CGACAGEGET
     601 GACACCACAG CTACACECAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
     SEL CICCGAGGC CCCITCITCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC
     721 CIGAGTERCE ARCIGGECCA AGECATERGT GACCACTATE CAGTGGAGGT GATGETGARG
     781 TGA
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pAS6 - light chain

```
LOCUS
            HMFG1LC2.D
                           721 bp
                                      DNA
            HUMANISED HMFG1 LIGHT CHRIN VND LEADER.
DEFINITION
ACCESSION
Keymords
SOUR/IE
  Drganiem
REFERENCE
                (BASES 1 TO 342)
  AUTHORS
            VERHOBYEN RI AL
            CONSTRUCTION OF RESHAPED EMFG1 ETC
  TITLE
            IMMUNOI. (1993):78, 364-370
  JOURNAL
            SCANNED IN FROM JOURNAL
COMMENT
FEATURES
  SITES
```

This is the sequence of the HMFG1 light chain gene with the Vnp leader sequence artached, Translate from residue 1. Note residue 399 in T > A in all clones leading to Al93 silent mutation (I in Verhoeyen paper)

BASE COUNT . 197 a 202 c 182 g 140 t ORIGIN LEWORL 1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCGAC 61 ATCCAGATGA CCCAGAGCCC AAGCAGCCIG AGCGCCAGCG TGGGTGACAG AGTGACCATC 121 ACCTGTAAGI CCAGTCAGAG CCIITTATAT ACTAGCAATC AAAAGATCIA CIIGGCCIGG 181 TACCAGCAGA AGCCAGGTAA GGCTCCAAAG CTGCTGATCT ACTGGGCATC CACTAGGGAA 241 TOTOGTGTGC CANGCAGATT CAGCGGTAGC GGTAGCGGTA COGACTTCAC CTTCACCATC 301 AGCAGCCTCC AGCCAGGA CATCGCCACC TACTACTGCC AGCANIATTA TAGATATCCT 361 EGGACGITCS GCCAAGGGAC CAAGGTCCAA ATCAAACCAA CTGTGGCTGC ACCATCTGTC 421 TICATCITCC CGCCATCTCA IGAGCAGIIG ARRICIGGAR CIGCCICICI TGIGIGCCIG 481 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGGCCTCCAA 541 TCCCCTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCAC CTACAGCCTC 601 AGCAGCACCC TEACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA

661 GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTIAG

"

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- Figure 3
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PAS6 - heavy chain (3)

```
LOCUS
             HHMFGIHC.D
                          1404 bp
                                     DNA
Definition
            HUMANISED HMFG1 heavy chain
ACCESSION BHILL GIR
KEYWORDS
SOURCE
  ORGANISM
REFERENCE
  AUTHORS
            VERHOETEN BT AL
  TITLE
            CONSTRUCTION OF RESHAPED EMFG1 atc
  JOURNAL
            IMMUNOL. (1993):78, 364-370
COMMENT
            WH domain SCANNED IN FROM JOURNAL
            AA RESIDUE 235 HAS NOT BEEN CHANGED TO KADAT (I.E. V TO A)
PEATURES
PEATURES
            Residue 963 is G > T leading to silent motation in all clones
  e3118
BASE COUNT
                333 -
                         439 c
                                  379 g
                                           253 t
ORIGIN
                 7 .
                                LEFPER .
       1 ATGGGATGGA GETGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GIGCAGCICG ICCACTCIGG GCCAGAGGIG AAAAAGCCIG GGGCCTCAGI GAAGGIGICC
     121 TOCAAGGCTI CIGGCTACAC CITCAGTGCC TACIGGATAG AGTGGGTGCG CCAGGCTCCA
     181 GGARAGGGCC ICGACTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC IAGATACAAT
     241 CAGAAGITCA AGGGCCGAGT GACAGICACT AGAGACACAT CCACAAACAC AGCCIACATG
     301 GAGCTCAGCA GCCTGAGGTC IGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
     361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGCTGA CAGTCTCCTG AGCCTCCACC
     421 AAGGGCCCAT CGGTCTTCCC CCIGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
     481 SCCCTGGGCT SCCTGGTCAR GGACTACTIC CCCGARCCGG TGACGGTGTC GTGGAACTCR
     541 GGCGCCTGA CCACCGCCT CCACCCTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
     601 TCCCTCAGCA GCGIGGIGAC CGICCCCTCC AGCAGCTIGG GCACCCAGAC CTACATCIGC
     661 RACGIBRATC ACANGCCCNG CHACACCANG GTCGRCARGA RAGTIGRECC CARATCTICT
     721 GACABAACIC ACACATGCCC ACCGTGCCCA GCACCTGAAL TCCTGGGGGG ACCGTCAGTC
     781 ITCCTUTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
     841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
     901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
     961 CETETEGICA GEGTECTELAC COTECTECAC CAGGACTEGE TGARTEGECAA GEAGTACAAG
    1021 TGCAAGGTCT CCAACAAAGC GGTGCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
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1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CIGCCCCCAI CCCGGGATGA GCTGACCAAG 1111 ANCCAGGICA GECTGAÇÇIG CCIGGICAAA GGCITCIAIC CCAGCGACAT CGCCGIGGAG 1201 TGGGAGAGCA ATGGGCAGCE GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 CACGECTECT TETTECTETA CAGCAAGETE ACCGTGGACA ACAGCAGGTG GCAGCAGGGG 1321 AACCTCTTCT CATGCTCGGT GATGCATGAG GCTCTGCACA ACCACTACAC GCACAAGAGC

* ANTIBUDY DIVAGE FUSIONS Maile MERRE (34-)39.)

hinge region HEAVY CHAIN Fab's Fusions were Made at this

Those with MYBRIB HINGES ARE MITERED

THIS PART GACAAAACTGACACA

1381 CTCTCCCTGT CTCCGGGTAR NIGA

THIS SEQUENCE YOU GET MYBRID HINGE & LINKER SEQUENCES Thon DWAZE I

FIGURE 9

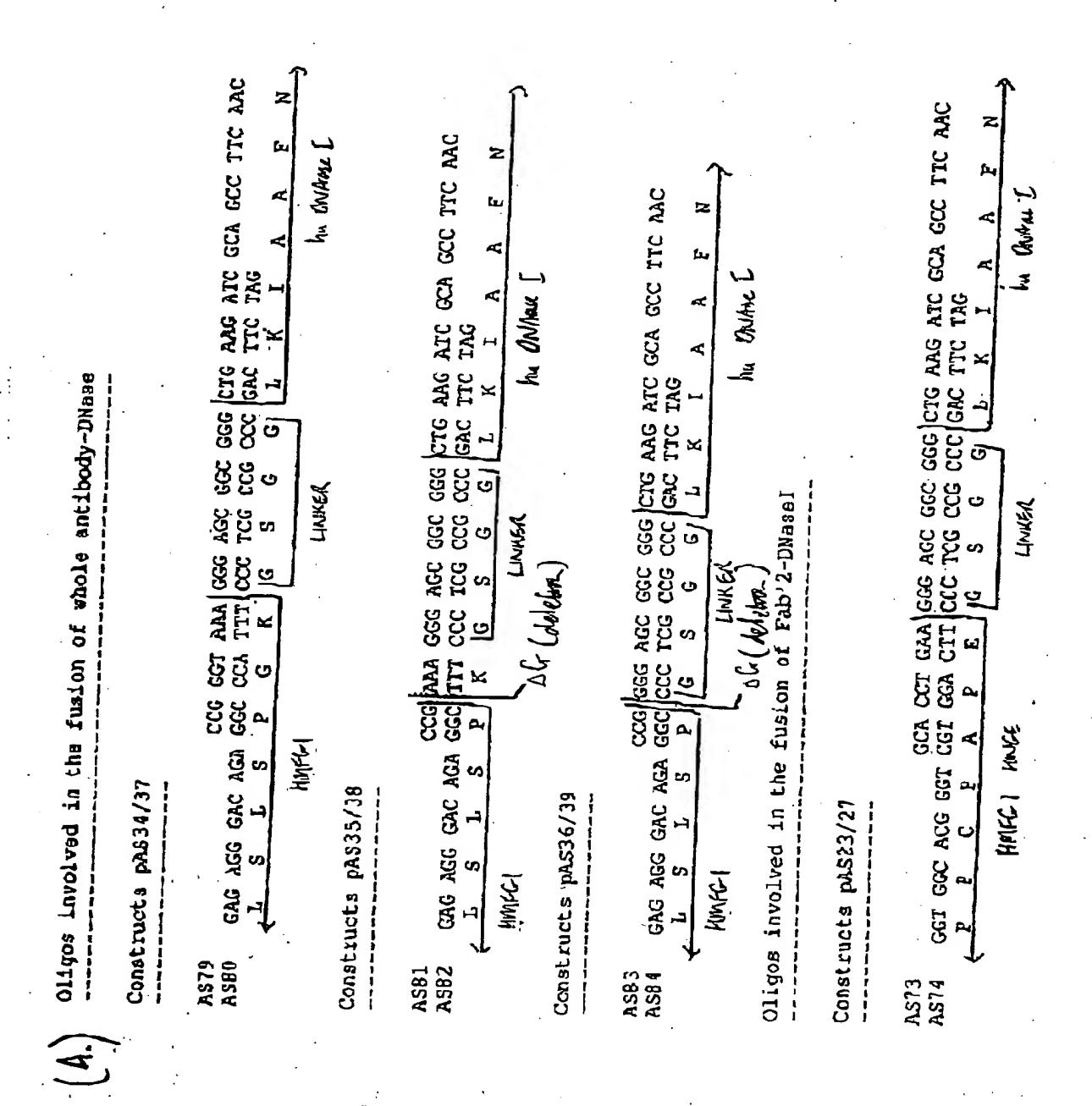
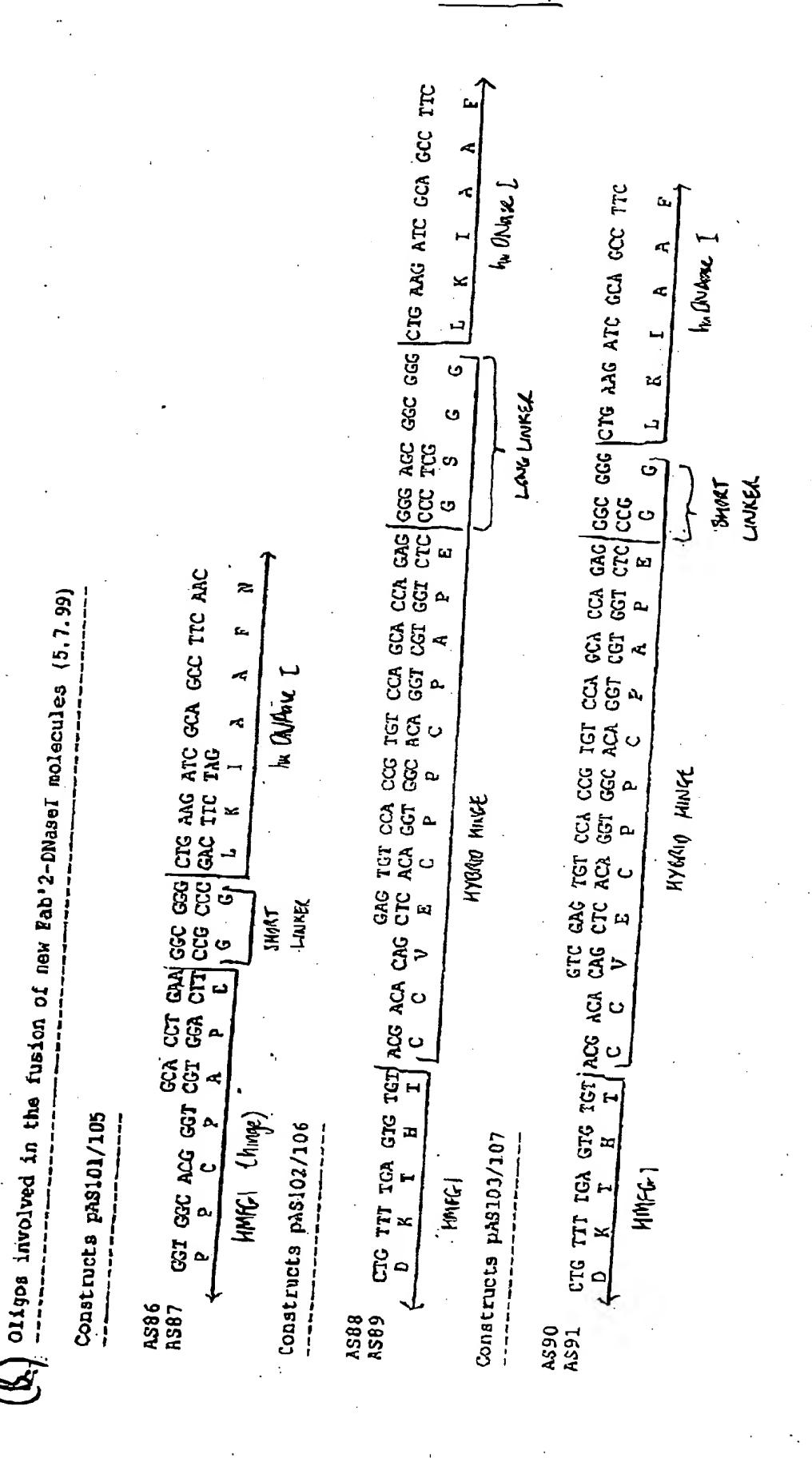
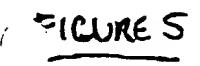


FIGURE 4



PAS23. DNA



LOCUS

pAS23

1554 bp mRNA PRI 06-MAR-1995 Humanised HMFG1 Fab'2 fused to human DNase I (construct 1) DEFINITION ACCESSION NID KEYWORDS DNase I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) Homo sapiens ORGANISM Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL MEDLINE 91067672 BASE COUNT 344 a 468 c 434 g - 308 t ORIGIN

1 ATCGCATGCA CCTCTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC 841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG 1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC 1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA 1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG 1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC 1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC 1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA 1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG 1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC 1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GTGA

File: PAS23 DNA
Range: 1 - 1554
Codon Table: Universal

Mode : Normal

FIGURE 5 (B)

	W 63 GTG V 117 CTC V 171 CGC R 225 AAT -N 279 ACA T 333	CAG Q TCC S TCC S TCC S	CTG TGC C TCT A TCT S	72 GTG V 126 AAG K 180 CCA P 234 AGA R	I CAG	TCT S AAG K AAT N	F 81 GGG G 135 GGC G 243 GAG E 297 TAC	GCA A TAC Y CTC L AAG K	GTA V GAG E ACC T GAG E TTC F	A 90 GTG V 144 TTC F 198 TGG W 252 AAG K 306	ACA T AAA K AGT S GTC V GGC GGC G	AAG AAG K GCC A GGA G CGA R	7 99 CCT 153 TAC Y 207 GAG V 315	GGT GGG GGG W ATT I ACA T AGG	CCC A ATA I TTA L GTC V	H 108 TCA S 162 GAG E 216 CCT P 270 ACT T
	W 63 GTG V 117 CTC V 171 CGC R 225 AAT -N 279 ACA T 333	CAG Q TCC S TCC S TCC S	CTG L TGC C A TCT S ACA	72 GTG V 126 AAG K 180 CCA P 234 AGA R 288 AAC	CAG CAG Q GCT A GGA GGA TAC Y ACA	TCT S AAG K AAT N GCC	F 81 GGG G 135 GGC G 243 GAG E 297 TAC	GCA A TAC Y CTC L AAG K	GAG T GAG T T T T T T T T T T T	A 90 GTG V 144 TTC F 198 TGG W 252 AAG K 306	AAA K AGT S GTC V GGC GGC G	AAG AAG K GCC A GGA G CGA R	7 99 CCT 153 TAC Y 207 GAG V 315	GGG GGG W ATT I ACA T AGG	CCC A ATA I TTA L GTC V TCT	H 108 TCA S 162 GAG E 216 CCT P 270 ACT T 324 GAG
	63 GTG V 117 CTC R 225 AAT -N 279 ACA T	CAG Q TCC S CAG N TCC S	CTG L TGC C A TCT S ACA	72 GTG V 126 AAG K 180 CCA P 234 AGA R 288 AAC	CAG Q GCT A GGA TAC Y ACA	TCT S AAG K AAT N GCC	81 GGG G 135 GGC G 243 GAG E 297 TAC	GCA A TAC Y CTC L AAG K	GAG T GAG T T T T T T T T T T T T T T T	90 GTG V 144 TTC F 198 TGG W 252 AAG K	AAA K AGT S GTC V GGC GGC G	AAG K GCC A GGA G	99 CCT \$ 153 TAC Y 207 GAG V 315	GGG GGG TGG W ATT I ACA T AGG	GCC A ATA I TTA L GTC V TCT	108 TCA S 162 GAG P 270 ACT T 324 GAG
	GTG V 117 CTC V 171 CGC R 225 AAT N 279 ACA T	Q TCC S	TGC C CCT A TCT S ACA	V 126 AAG K 180 CCA P 234 AGA R 288 AAC	CAG Q GCT A GGA Y ACA	TCT S AAG K AAT N GCC	GGG G 135 GGC G 169 GGC G 243 GAG E 297 TAC	GCA A TAC Y CTC L	ACC T GAG TTC F	GTG V 144 TTC F 198 TGG W 252 AAG K	AAA K AGT S GTC V GGC GGC G	GCC A GGA GGA CGA R	CCT P 153 TAC Y 207 GAG V 315	GGG C TGG W ATT I ACA T AGG	ATA I TTA L GTC V TCT	TCA S 162 GAG E 216 CCT P 270 ACT T 324 GAG
	V 117 CTC V 171 CGC R 225 AAT N 279 ACA T	Q TCC S	TGC C CCT A TCT S ACA	V 126 AAG K 180 CCA P 234 AGA R 288 AAC	GCT A GGA TAC Y ACA	S TOT S AAG K AAT N	G 135 GGC G 243 GAG E 297 TAC	TAC TAC Y CTC L AAG	ACC T GAG TTC F	V 144 TTC F 198 TGG W 252 AAG K	AGT S GTC V GGC GGC G	GCC A GGA GGA CGA R	153 TAC Y 207 GAG E 261 GTG V	TGG W ATT I ACA T	ATA I TTA L GTC V TCT	S 162 GAG E 216 CCT P 270 ACT T 324 GAG
	117 CTC V 171 CGC R 225 AAT N 279 ACA T	TCC S	TGC C GCT A TCT S ACA	126 AAG K 180 CCA P 234 AGA R 288 AAC	GCT A GGA GCA TAC Y ACA	TCT S AAG K AAT N	135 GGC G 169 GGC G 243 GAG E	TAC Y CTC L AAG	ACC T T GAG E TTC	144 TTC F 198 TGG W 252 AAG K	AGT S GTC V GGC GGC G	GCC A GGA G CGA R	153 TAC Y 207 GAG E 261 GTG V	TGG W ATT I ACA T AGG	ATA I TTA L GTC V TCT	162 GAG E 216 CCT P 270 ACT T 324 GAG
	TC V 171 CGC R 225 AAT N 279 ACA T 333	CAG Q AAT N TCC S	C GCT A	AAG K 180 CCA P 234 AGA R 288 AAC	GCT A GGA GGA TAC Y ACA	AAG K AAT N	GGC G 169 GGC G 243 GAG E	TAC Y CTC L AAG	GAG E TTC	TTC F 198 TGG W 252 AAG K	AGT S GTC V GGC GGC G	GGA GGA CGA R	TAC Y 207 GAG E 261 GTG V	TGG W ATT I ACA T AGG	TTA L GTC V TCT	GAG E 216 CCT P 270 ACT T 324 GAG
	7 171 CGC R 225 AAT N 279 ACA T	CAG Q AAT N TCC S	C GCT A	180 CCA P 234 AGA R 288 AAC	GGA GA TAC Y	AAG K AAT N	GGC G 169 GGC G 243 GAG E	TAC Y CTC L AAG	GAG E TTC	TTC F 198 TGG W 252 AAG K	AGT S GTC V GGC GGC G	GGA GGA CGA R	TAC Y 207 GAG E 261 GTG V	TGG W ATT I ACA T AGG	TTA L GTC V TCT	GAG E 216 CCT P 270 ACT T 324 GAG
	171 CGC R 225 AAT N 279 ACA T	CAG Q AAT N TCC S	GCT A TCT S	180 CCA P 234 AGA R 288 AAC	GGA GGA TAC TAC Y	AAG K AAT N	169 GGC G 243 GAG E 297 TAC	CTC L AAG	GAG E TTC	198 TGG W 252 AAG K	GTC V GGC G	GGA G CGA R	207 GAG E 261 GTG V	ATT I ACA T AGG	TTA L GTC V TCT	216 CCT P 270 ACT T
	CGC R 225 AAT N 279 ACA T	Q AAT N TCC	A TCT	180 CCA P 234 AGA R 288 AAC	GGA GGA TAC TAC Y	AAG K AAT N	169 GGC G 243 GAG E 297 TAC	CTC L AAG	GAG E TTC	198 TGG W 252 AAG K	GTC V GGC G	GGA G CGA R	207 GAG E 261 GTG V	ATT I ACA T AGG	TTA L GTC V TCT	216 CCT P 270 ACT T
	CGC R 225 AAT N 279 ACA T	Q AAT N TCC	A TCT	P 234 AGA R 288 AAC	GGA GGA TAC TAC Y	K AAT N GCC	GGC G 243 GAG E 297 TAC	L AAG K	GAG E TTC	TGG W 252 AAG K	V GGC G	G CGA R	GAG E 261 GTG V	ATT I ACA T AGG	GTC V TCT	CCT P 270 ACT T 324 GAG
	R 225 AAT N 279 ACA T	Q AAT N TCC	A TCT	P 234 AGA R 288 AAC	TAC Y	K AAT N GCC	G 243 GAG E 297 TAC	L AAG K	E TTC F	Z52 AAG K	V GGC G	G CGA R	261 GTG V	I ACA T	GTC V TCT	270 ACT T 324 GAG
	225 AAT N 279 ACA T	AAT N TCC	TCT s	234 AGA R 288 AAC	TAC Y ACA	AAT N GCC	243 GAG E 297 TAC	AAG K	TTC F	252 AAG K	GGC G	CGA R	261 GTG V	ACA T	GTC V TCT	270 ACT T 324 GAG
	AAT N 279 ACA T	AAT N TCC	S ACA	AGA R 288 AAC	TAC Y ACA	AAT N GCC	GAG E 297 TAC	AAG K	TTC F	AAG K 306	GGC G	CGA R	GTG V	ACA T	GTC V TCT	ACT T 324 GAG
2 3 3 3	N 279 ACA T	TCC	S ACA	AGA R 288 AAC	TAC Y ACA	AAT N GCC	GAG E 297 TAC	AAG K	TTC F	AAG K 306	GGC G	CGA R	GTG V	ACA T	GTC V TCT	ACT T 324 GAG
2 SC 7 S	279 ACA T	TCC S	ACA	288 AAC	ACA	GCC	297 TAC			306		,	315	λGG	TCT	324 GAG
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-	4 2 0 	495 CTG L 549	495 CTG GTC L V	495 CTG GTC AAG L V K	495 504 CTG GTC AAG GAC L V K D 549 558	495 504 CTG GTC AAG GAC TAC L V K D Y 549 558	495 504 CTG GTC AAG GAC TAC TTC L V K D Y F 549 558	495 504 513 CTG GTC AAG GAC TAC TTC CCC L V K D Y F P 549 558 557	495 504 513 CTG GTC AAG GAC TAC TTC CCC GAA L V K D Y F P E 549 558 567	495 504 513 CTG GTC AAG GAC TAC TTC CCC GAA CCG L V K D Y F P E P 549 558 557	495 504 513 522 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG L V K D Y F P E P V 549 558 557 576	495 504 513 522 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG L V K D Y F P E P V T 549 558 567 576	495 504 513 522 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG L V K D Y F P E P V T V 549 558 567 576	495 504 513 522 531 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG L V K D Y F P E P V T V S 549 558 567 576 585	495 504 513 522 531 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG L V K D Y F P E P V T V S W 549 558 557 576 585	495 504 513 522 531 CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC L V K D Y F P E P V T V S W N

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	~			CTG					AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG
Y .	D	I	A	L	V	Q ,	E	V	R	D	s	н	L	T	A	v	G
220	C=0	927			936			945			954			963			972
			GAC	AAC	CTC	AAT 	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GTG	GTC
K	L	L	D	N	L	N	Q	ם	A	P	D	т	Y	н	X.	v	v
		981			990			999		1	800		3	017		1	026
AGT	GAG	CCA	CTC	CCA	CGG .	AAC	AÇC	7AT 	AAG	GAG	CGC	TAC	CTG	TTC	GTC	TAC	ACC.
S	E	Þ	L	G	R	N	S	Y	ĸ	E	R	Y	Ľ	F	v	У	 R
		1035		1	044		1	053		1	062		1	071		1	080
CCT	GAC	CAG	GTG	TCT	GCG (GTG	GAC .	AGC	TAC	TAC	TAC	gat	GAT	GGC	TGC	GAG	ccc
P	D	Q.	V	s	A	ν	ס	S	Y	Υ	Y	ם	D	G	 C	 E	P
maa.		1089		1	098		1:	107		1	115		1	125		1	134
TGC	GGG 	AAC	GAC	ACC (TTC 1	AAC (CGA (GAG (ICA ·	GCC .	ATT	GTC .	AGG	TTC	TTC	TCC (CGG
Ċ	G-	N	D	T	F	Ŋ	R	E	P	A	I	v	R	F	F	s	R
		143		1:	152		1:	161		1 .	170		1	179		1:	188
TTC	ACA	GAG	GTC	AGG (GAG 1	TTT (GCC A	ATT (STT (CCC (CTG	CAT	GCG	GCC	CCG	GGG (SAC
F	T	E	V	R	E	F	A	 I	ν	P	L	- - -		Λ	 P	с С	D
	1	197		12	206		12	215		1:	224		1	233		12	242

GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GÀT GTC CAA GAG AAA AVAEIDALYDVQ 1251 1259 1278 1287 1260 1296 TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT WGLEDVMLMGDFNAGC 1305 1314 1323 1332 1341 GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG V R P S Q W S S I R L . W T S P T F Q 1359 1368 1377 1386 1395 TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT WLIPDSADTTATHCAY 1413 1422 1431 1440 1449 1458 GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG D R I V V A G M L L R G A V V P D S 1457 1475 1485 1494 1503 GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA ALPFNFQAAYGLSDQLAQ 1521 1530 1539 1548 GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3' A I S D H Y P V E V M L K *

FIGURE 6

(A) pAS27

LOCUS PAS27. DNA 1584 bp mRNA PRI 06-MAR-1995 DEFINITION Humanised HMFGl Fab'2 fused to human DNase I with SV40 NLS (construct 1) ACCESSION NID KEYWORDS DNase I. SOURCE DNase I sequence is from assembled oligos (thus modified c/f MHDNASEl.dna) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL 91067672 MEDLINE BASE COUNT 354 a474 c 446 g 310 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TITGCCTGGT TIGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATU AUAAGGUUAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC 781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC 841 TACATTGTCC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC 901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT 961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG 1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC 1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA 1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG 1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGGCTT GGAGGACGTC 1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC 1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA 1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG 1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC 1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GCGCGCGCGA 1561 CCCAAAAAGA AGCGCAAGGT TTGA

L3 NLS

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File: PAS27.DNA
Range: 1 - 1584
Codon Table: Universal

Mode : Normal

FIGURE 6 (B)

,	ATG	GGZ	9 A TGO		TGI	18 ATC		CTC	27		:	36 4 GC2		CCT	45 מרא י		ር ው	54 CAC
															. ACA			CAC
	M	G	W	S	С	I	I	L	F	L	V	A	T	A	T	G	V	H
	me e		63			72			81						99			108
		LAC	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA
	S	Q	V	Q	L	V	Q	S	G	A	E	V	к	K	P	G	A	s S
			117	•		126			135			144			153			162
	GTG	AAG	GTG	TCC	, TGC	AAG								GCC	TAC	TGG	ATA	GAG
	v	ĸ	v	 5	c	ĸ	 A	 5		 Y		 F	 S	 A	Y		~-~ I	
			484				•	-		• .	•				*	NA		E
	TGG	GTG	171 CGC			180		באמ	189 GGC	כיתיכי	CAC	198	cme	CCN	207 GAG	3.00		216
				-							ong	166		GGA	GAG	ATT	TTA	CCT
	W	٧	R	Q.	A	P	G	K	G	L	E	W	v	G	E	I	L	P
	_		225			234			243			252			261			270
	GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	ĸ	 F		G	 R	V.	 T		 T
			279			288			297	, ·		306			245			
	AÇA	CAC		TCC	ACA		ACA	acc		ATC	CAC	306 CTC		AÇC	315 crc	ACC	TCT	324 CAC
	 R	 D	T	 S	 Т		 Т	 A		 M	 E							
					_	_ `	•	***	*	P1	<i>-</i>	L	S	2	L	R	8	E
	GAC	ACA	333 GCC	GTC	ጥልጥ	342 TAC	ጥርጥ	GCA	351 NGD	TCC	3 5.0	360			369 TGG			378
												GAC			TGG	TTT	GCT	TAC
	D	T	A	V	Y	Y	C	A	R	\$	Y	D	F	A	W	F	A	Y
			387			396			405			414			423	,		432
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TÇG
	W	G	Q	G	T	L	V	T	v	s	- - -	 A	s	 T	K	G	P	S
			441			450			459			468			477			
	GTC	TTC		CTG	GCA		TCC	TCC		AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	486 CTG
	v	 F	 P	 L	 A	 P	 \$		 K	 s	 T	 s	- G					
							_	_		-	•	J	G	G	T	A	A	L
	GGC	TGC	495 CTG	GTC	AAG	504 GAC	TAC		513 CCC	CAA	ררכ	522	3 C C	CTC	531 TCG	* ******		540 Bar
																166	AAC	TCA
	G	C	L	V .	K	D	Y	F	P	E	P	V	T .	V	S	. W	N	S
	A	<i></i>	549			558			567			576	•	·	585			594
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	acċ 	TTC	CCG	GCT	GTC	CTA	CAG	TÇÇ	TCA	CCA
	G	A	L .	T	S	G	V	Н	T	F	P	A	V	L L	Q	s	s	 G
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603 612 621 630 639 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG LYSLSSVV S S L G T O T V P
 565
 675
 684
 693
 702
 657 ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSNTKVDKK 711 720 729 738 747 756 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT V E P K S C D K T H T C P P C P A P 774 783 792 801 810 765 GAA GGG AGC GGC CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG E G S G G L K I A A F N I Q T F G E 828 837 846 855 819 ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TKMSNATLVSYIVQILSR 873 882 900 909 891 918 TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG YDIALVQEVRDSHL 936 945 927 954 963 972 AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC K L L D N L N-Q D A P D T Y H Y V 981 990 999 1008 1017 1025 AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG G R M S Y K ERY 1035 1044 1053 1062 1071 1080 CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC D Q V S A V D S Y Y Y D D 1089 1098 1107 1116 1125 1134 TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TCC CGG CGNDTFNREPAIVRFFSR 1143 1161 1170 1152 1179 1188 TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT CCC CCC CCC CCC CAC TEVREF AIVPLH AAPGD 1197 1206 1215 1224 1233 1242

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GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA AVAEIDALYDVY 1260 1269 1278 1287 1251 TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT WGLEDVMLMGDFNAGCSY 1305 1314 1323 1332 1341 1350 CTG AGA CCC TCC CAG TGG TCA TCC ATC CCC CTC TCG ACA AGC CCC ACC TTC CAG V R P S Q W S S I R L W T S P T F Q 1359 1368 1377 1386 1395 1404 TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT WLIPDSADTTATPTHCAY 1413 1422 1431 1440 1449 CAC AGG ATC GTG GTT GCA GGG ATG CTC CTC CCA CCC CCC GTT GTT CCC GAC TCG DRIVVAGMLLRGAVVFDS 1467 1476 1485 1494 1503 1512 GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA ALPFNFOAAYGLSDQLAQ 1521 1530 1539 1548 1557 1566 GCC ATC AGT GAC CAC TAT CCA CTC CAC CTC ATC CTG AAG GGG GGC GGA CCC AAA AISDHYPVEVMLKGGGPK 1575 1584

AAG AAG CGC AAG GTT TGA 3' K K R K V *

FIGURE 7

(A) pAS34

LOCUS PAS34.DNA 2196 bp 2196 bp 2196 bp DNA DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse construct 34 Clone 16.4.2 (same as hcdnasel.dna template file) DEFINITION REFERENCE AUTHORS VERHOEYEN ET AL TITLE CONSTRUCTION OF RESHAPED HMFG1 etc JOURNAL IMMUNOL. (1993):78, 364-370 Human DNAse sequence is modified as a result of oligo assembly COMMENT (mhdnase.dna) The fusion was made using overlapping oligos AS79 and AS80 COMMENT FEATURES AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) Residue 963 is G > T leading to silent mutation in all clones FEATURES SITES Note BASE COUNT 501 a 677 c 607 g 411 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 651 AAUGTGAATU ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 791 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TCCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG 1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG 1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG 1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG 1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG 1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC 1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA GAGAGGTCAG GGAGTTTGCC 1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC 1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC 1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC 1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT 2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG 2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC 2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGTGA

File : PAS34.DNA 2196 Mode: Normal Range: 1 -FIGURE 7 (B) Codon Table : Universal 18 27 36 ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC M G I L F V 72 81 90 99 108 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA Q S G A E VKKP 117 126 144 153 135 162 GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG ĸ S G Y T F S 171 180 189 198 207 216 TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT WVRQAP G K G L 225 234 243 252 261 270 GGA AGT AAT AAT TOT AGA TAC AAT GAG AAG TTO AAG GGO CGA GTG ACA GTO ACT K F K S R Y N E 279 288 297 306 315 324 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG NT A Y M E 5 333 342 351 360 369 378 CAC ACA CCC CTC TAT TAC TCT CCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC YCARSY D F 387 396 405 414 423 432 TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG 5 A 5 441 450 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG 5 ĸ 5 495 504 513 **522** 531 540 С K D P E 549 558 567 576 585 594 GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA G A S G V H 503 612 621 530 639 648 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG

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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNH.KPSNTKVDKK 711 720 729 738 747 756 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT V E P K S C D K HTCPPCPAP T 774 783 792 801 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT 819 828 937 846 855 964 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC LMISRTPEVTCVVDVSH 873 882 591 900 909 918 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT EAKEN WYVDGVEVHN 927 936 945 954 963 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC AKTKPREEQYMSTYRVVS 981 990 999 1008 1017 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N G K E Y K C K 1035 1044 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T I S K A K 1098 1107 1089 1116 1125 1134 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG G Q P R E P Q V Y T L P P S R D 1143 1152 1161 1170 1179 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC TK, NQ VSLTCLVKGFYPSD 1197 1206 1215 1224 1233 1242 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG IAVEWESNCQPENNYK 1251 1260 1269 1278 1287 1296 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPV'LDSDGSFFLYSKLTV 1305 1314 1323 1332 1341 1350 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG

D K 5 R W Q Q G N V F S C S V M H E

03/04 '00 MON 14:40 FAX 01159 552201 ERIC POTTER CLARKSON 1359 1368 1377 1396 1395 GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTC TCT CCC CCT AAA CCC A L H N H Y T O K S L S P G K G 1413 1422 1431 1440 1449 AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG S G G L K I A A P N I Q T F G E T K 1467 1476 1485 1494 1503 1512 ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC M S N A T L V S Y I V Q I L S R Y D 1521 1530 1539 1548 1557 ATC CCC CTC CTC CAG CAG CTC AGA CAC AGC CAG CTG ACT GCC GTG GGG AAG CTG I A'L V O E V R D S H L T A V G K L 1575 1584 1593 1602 1611 CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG L D N L N Q D A D D T Y H Y V S E 1638 1647 1656 1665 1674 1629 CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC P L G R N S Y K E R Y L F V Y R P D 1692 1701 1683 1710 1719 CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG Q V S A V D S Y Y Y D D G C E P C G 1746 1755 1764 1737 1773 AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA NDTFNREPAIVRFFFT 1809 1818 1827 1836 1791 1800 GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA E V R E F A I V P L H A A P G D A V 1854 1863 1872 1845 1881 1890 GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC A E I D A L Y D V Y L D V Q E K 1908 1917 1926 1935 1944 1899 TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT, GCG GGC TGC AGC TAT GTG AGA LEDVMLMGDFNAGCSXVR 1953 1962 1971 1980 1989 1998 CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG PSQWSSI R L W T S P 2007 2016 2025 2034 2043 2052 ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG

P D S A D T T A T P T H C A Y D R

2061 2070 2079 2088 2097 21.06 ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT I V V A G M L L R G A V V P D S A L 2115 2124 2133 2142 2151 2160 CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC PFNFQAAYGLSDQLAQAI. 2169 2178 2187 2196 AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

LOCUS

FIGURE 8

PAS35.DNA 2193 bp 2193 bp DNA 14-AUG-1998 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse construct 35 Clone 17.12.1 with silent K to K mutation (1398 A > G) DEFINITION REFERENCE AUTHORS VERHOEYEN ET AL CONSTRUCTION OF RESHAPED HMFG1 etc TITLE JOURNAL IMMUNOL. (1993):78, 364-370 Human DNAse sequence is modified as a result of oligo assembly COMMENT (mhdnase.dna) The fusion was made using overlapping oligos AS81 and AS82 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) FEATURES FEATURES Residue 963 is G > T leading to silent mutation in all clones In 17.12.1 residue 1398 is A > G (silent K to K mutation) FEATURES SITES Note BASE COUNT 500 a 677 c 606 q 410 t ORIGIN

1 ATEGGATEGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGGAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAC CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA 1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC 1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG 1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA 1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT 1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC 1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT 1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC 1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT 1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC 1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC 2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT 2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT 2161 GACCACTATC CAGTGGAGGT GATGCTGAAG TGA

Fila: PAS35.DNA
Range: 1 - 2193
Codon Table: Universal

Mode : Normal

FIGURE 8(B)

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5,	ATG	Gga	e Dot <i>i</i>			. 18											GTC	54 CAC
	M	G	W	 S				 L	 F	L L		 A	 T	 A	 T			
	TCC	CAG	63 GTG		ርጥር	72 GTG	CAG	ጥረጭ	81	COX	CAC	90		330	99	,		108 TCA
						~~										GGG 	GCC	TCA
	3	· Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S
	GTG	AAG	117 GTG		TGC	AAG	GCT	TCT	135 GGC	TAC	ACC	144 TTC	AGT	GCC	153 TAC	TGG	АТА	162 GAG
	v	K	v	\$	C	ĸ	A	S	G	Y	T	F	S	A	Y	W	I	E
	TGG	GTG	171 CGC		GCT	180 CCA	GGA	AAG	189 GGC	CTC	GAG	198 TGG	GTC	GGA	207 GAG	ል ጥጥ	ሊ ተነጥ አ	216 CCT
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	γV	V	R	Q	A	P	G	K	æ	I.	E	W	ν	G	E	I	L	~ P
	GGA	AGT				234				33C				553				270 ACT
												AAG		CGA	GTG	ACA	GTC	ACT
	G	5	N	N	S	R	¥	N	E	K	F	K	G	R	V	T	V	T
	202	c) c	279												315			324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	s	ֹז	N	T	A	¥	M	E	L	S	S	L	R	5	E
	GAC	ACA	333 GCC	GTC	ТАТ	342 TAC	TGT	GCA	351	ጥርር	መልሮ	360		^~~		చచచ	~~~	378
																 		TMC
	D	T		V	Y	Y	C	A	R	S	Y	D	F	A	M	F	A	Y
	TICC	CCC	387	204) // (m	396	oma	101	405			414	_		423			432
					AUT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA.	TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	Т	K	G	. ?	S
•			441			450			459			468			477			486
	GTC	TTC	ccc	CTG	GCA	ccc	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
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			495			504			51 2			577			637			5.40
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	CYY	CCG	CTC	ACG	CTC	TCC	TCC	AAC	540 TCA
	G	С	r	v	ĸ	D	Y	F	Þ	E	P	v	 T		 5	w		 s
		•	549			558			567			576		•	585			5 Q /I
	GGC	GCC														TCC		
	G	A	L	T	s	G	v	н	T	 F	P	-	v	 L	Q	S	 s	G
			603		•	612			621			630			639			648
	CTC	TAC	TĊĊ	CTC	AGC	AGC	GTG	GTG	ACC	GTG	ccc	TCC	AGC	AGC	TTG	GGC	ACC	CAG
	L	Y	s	L	S	s	v	v	T	v	p p	 s	 s	 s		c	 T	Q
			657			666			675			684	٠		693			702
												•						

03/04 '00 MON 14:41 FAX 01159 552201 ERIC POTTER CLARKSON ACC TAC ATC TOC AAC OTG AAT CAC AAG CCC ACC AAC ACC AAC CTC CAC AAG.AAA T Y I C N V N H K P S N T K V D K K 738 711 720 729 747 756 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT V E P K S C D K T H 765 774 783 792 801 810 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKD 819 828 837 846 955 864 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC MISRTPEVTCVVV 873 882 891 900 909 918 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT EVKFNWYVDGVEV 927 936 945 954 963 972 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC E E AKTKPR Q Y N S T Y R 981 990 ōōō 1008 1017 7026 CTC CTC ACC CTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V ~L T V · L H Q D W L N G K E Y K C K 1035 1044 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T ISKAK 1089 1098 1107 1115 1125 1134 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG R G 1152 1161 1143 1170 1179 1188 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC T K N Q V S L T C L V K G F Y P 1206 1215 1224 1233 1242 1197 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG I A V E W E S N G Q P E N N Y K 1269 1278 1251 1260 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDGS.FFLY SKL 1314 1323 1332 1305 1341 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG

- 2 -

D K S R W Q Q G N V F S C S V M H E

1368 1377 1359 1386 1395 GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC A L H N H Y T Q K S L S P K G S 1413 1422 1431 1440 1449 GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG G G L K I A A F N I Q T F C F T K M 1467 1475 1485 1494 1503 1512 TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC SNATLVSYIVQILSRY 1521 1530 1539 1548 1557 GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG A L V Q E V R D S H L T- A V G K L L 1575 1611 CAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA D. M F M O D Y D D A A A A A 1529 1638 1647 1656 1565 CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG LGRNSYKERYLFVYRPDQ 1683 1701 1710 1692 1719 1728 GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC V S A V D S Y Y Y D D G C E P C G N 1737 1746 \ 1755 1764 1773 1782 GAC ACC TTC AAC CCA GAC CCA GCC ATT GTC AGG TTC TCC CGG TTC ACA GAG DTFN REPAIVRFFSRF_E 1800 1809 1818 1827 1836 1791 GTC AGG GAG TIT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC V R E F A I V P L H A A P G D A V A 1845 1854 1863 1872 1881 1890 GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG EIDALYDVYLDVQEKWGL 1899 1908 1917 1926 1935 GAG GAC GTC ATC TTC ATC CCC CAC TTC AAT CCC GGC TGC ACC TAT GTC AGA CCC D F N L M G A G C 5 Y V R P 1953 1962 1971 1980 1989 1998 TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC S Q W S S I R L W T S P T F Q W L I 2007 2016 2025 2034 2043 CCC GAC AGC GCT GAC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC

TTATPTHCAYDRI

S A

2061 2070 2079 2088 2097 2106

GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC

V V A C M L L R G A V V P D S A L P

2115 2124 2133 2142 2151 2160

TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT

F N F Q A A Y G L S D Q L A Q A I S

GAC CAC TAT CCA GTG GAC CTC ATC CTC AAC TCA 3'

D H Y P V E V M L K *

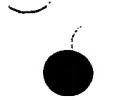


figure 9

(1) pAS36

PAS36.DNA LOCUS 2190 bp 2190 bp DNA 14-AUG-1998 HUMANISED HMFGl heavy chain fused to human DNAse - construct 36 DEFINITION Clone 18.24.1 with residue 1392 T > C DEFINITION REFERENCE AUTHORS VERHOEYEN ET AL CONSTRUCTION OF RESHAPED HMFG1 etc TITLE IMMUNOL. (1993):78, 364-370 JOURNAL Human DNAse sequence is modified as a result of oligo assembly COMMENT (mhdhase.dna) The fusion was made using overlapping oligos AS83 and AS84 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) FEATURES Residue 963 is G > T leading to silent mutation in all clones FEATURES Residue 1392 T > C silent S to S mutation FEATURES SITES Note BASE COUNT 678 c 605 g 498 a 409 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CcCCGGGGAG CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT 1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC 1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG 1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG 1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG 1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA 1741 GAGCCAGCCA TIGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT 1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC 1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG 1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC 1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT 2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT 2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC 2161 CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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File: PAS36.DNA
Range: 1 - 2190
Codon Table: Universal

Mode : Normal

FIGURE 9 (B)

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į	ATĠ	CCA	9 TCC										ACA					
	M	G	W	 S	c		 I	 L	 F	L L	v	 A	 T	A	T	G G	v	н
	TCC	CAG	63 GTG										AAA			GGG		108 TCA
	s	ð	v	Q	L		Q	s	G	A	E	v	K	K	P	G	A	s
1	GTG	AAG	117 GTG	TCC	TGC								AGT				ATA	162 GAG
	v	к	V	క	c	ĸ	A	3	G	¥	T	F	s	A	¥	VJ	I	E
	TGG	GTG	171 CGC	CAG	GCT								GTC			ATT	TTA	216 CCT
	M	V	R	Q	A	P	G	K	G	L	E	W	v	G	E	I	L	P
	GGA	AGT	225 AAT				•						GGC			ACA		
	G	s	N	Ŋ	s	R	Y.	N	E	ĸ	F	ĸ	G	R	v	T	V	T
	AGA	GAC	279 aca	TCC	ACA	288 aac	ACA	GCC		atg			AGC		315 CTG		тст	324 GAG
	R	D	T	3	T	M	T	A	Y	M	E	L	s	s	L	R	s	E
	CAC	ACA	333 GCC	GTC	TAT	342 TAC	TGT			TCC			TTT		369 TGG	TTT 	GCT	378 TAC
	D	T	A	ν	¥	Y	C	A	R	S	Y	D	F	A	W	F	A	¥
	TGG 	GGC	387 CAA		ACT	396 CTG		ACA					TCC		423 AAG		CCA	432 TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	ĸ	Ġ	P	S
	GTC	TTC	441 CCC	CTG		450 CCC					ACC		GG G	GGC	477 ACA		GCC	486 CTG
	V	F	P	r.	A	P	\$	S	ĸ	2	T	5	C	G	T	A	A	L
	GGC	TGC	495 CTG		AAG	504 GAC		TTC	513 ccc		CCG	522 GTG		GTG	531 TCG		AAC	540 TCA
	G	C	L	v	ĸ	D	¥	F	P	E	P	v	T	V.	\$	W	N	S
	GGC	GCC	549 CTG		AGC	558 GGC	•						GTC				TCA	594 GGA
	G	A	Ł	T	s	G	v	н	T	F	P	A	v.	L	Ø	S	s	G
	CTC	TAC	603 TCC		AGC	612 AGC							ACC				ACC	648 CAG
	L	Y	\$	L	S	S	· V	v	T	ν	P	S	S	S	L	G	T	Q
			657			666			675			584	ı		693			702
												- 1 -						



ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSNTKVDKK 711 720 729 738 747 756 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT A E B K S C D K T H T C P P 774 783 792 801 810 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC 5 V F PKPKDT F 828 837 846 **855** CTC ATG ATC TCC CGG ACC CCT CAC CTC ACA TCC CTC CTC CTC CAC CTC ACC CAC LMISRTPEVTC V V D V S H 873 882 891 900 909 918 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT VKF N W Y V 927 936 945 954 963 972 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC AKTKPREEQYN 981 990 999 1008 1017 1026 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N G K E Y K C K 1035 1044 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA A L P A P I E K T I S 1089 1098 1107 1116 1125 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG E P Q V Y T L 1143 1152 1161 1170 1179 1188 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC T K N Q V S L T C L V K G F Y P S D 1197 1206 1215 1224 1233 1242 ATC GCC GTG GAG TGG CAG ACC AAT CCC CAC CCC CAC AAC AAC TAC AAG ACC ACG IAVEWESNGOPENNYK 1251 1260 1269 1278 1287 1296 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDGSFFLYSKLTV 1305 1314 1323 1332 1341 1350 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG

D K S R W Q Q G N V F S C S V M H P

1368 1377 1386 1395 1359 GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCG GGG AGC GGC A L H N H Y T Q K S L S P G S G 1413 1431 1422 1440 1449 1458 GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC G L K I A A F N I Q T F G E T K M S 1467 1476 1485 1494 1503 1512 AAT GCC. ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC NATLVSYIVQILSRYDIA 1521 1530 1539 1548 1557 1566 CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG GAC LVQEVRDSHLTAVGKLLD 1575 1584 1593 1602 1611 1620 AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG N L N Q D A P D T Y H Y V V S E P L 1647 1629 1638 1636 1665 GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG GRNSYKERYLFVYRPDQV 1683 1692 1710 1701 1719 TCT GCG GTG GAC AGC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC GAC S A V D S Y Y Y D D G C E P C G N D 1737 1746 1755 1764 1773 ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC T FNREPAIVRFFSRFTEV 1791 1800 1809 1818 1827 AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG R E F H A A P G 1845 1854 1863 1872 1881 ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG GAG IDALYDVYLDVQEKWGLE 1908 1917 1926 1935 1944 1899 GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC D V M L M G D F N A G C S Y V R P S 1962 1971 1980 1989 1998 1953 CAG TGG TGA TGC ATC CGC CTG TGG ACA ACC CCC ACC TTC CAG TGG CTG ATC CCC W S S I R L W T S P T F Q W L I P 2007 2016 2034 2043 2052 2025 GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG

D S A D T T A T P T H C A Y D R I V

2079 2088 2097 2070 2106 2061 .GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT V A G M L L R G A V V P 2133 2142 2151 . 2160 2124 2115 AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC N F Q A'A Y G L S D Q L A Q A I S D

2178 . 2187 2169 CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3' E V M

FI CURE 10

(A) <u>pAS37</u>

LOCUS PAS37.DNA 2226 bp 2196 bp 2196 bp DNA HUMANISED HMFG1 heavy chain fused to human DNAse construct 37 DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file) plus NLS DEFINITION REFERENCE VERHOEYEN ET AL AUTHORS CONSTRUCTION OF RESHAPED HMFG1 etc TITLE JOURNAL IMMUNOL. (1993):78, 364-370 Human DNAse sequence is modified as a result of oligo assembly COMMENT (mhdnase.dna) The fusion was made using overlapping oligos AS79 and AS80 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) **FEATURES** Residue 963 is G > T leading to silent mutation in all clones FEATURES SITES Note BASE COUNT 683 c 511 a 619 q 413 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TECAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG 1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG 1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG. 1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG 1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG 1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC 1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC 1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC 1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC 1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC 1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT 2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG 2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC 2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGGGGGGGG GACCCAAAAA GAAGCGCAAG 2221 GTTTGA

LONG

11

5,

File: PAS37.DNA
Range: 1 - 2226
Codon Table: Universal

2226 Mode : Normal

FIGURE 10 (B)

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-			9															
•	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	W	ຣ ່	С	I	I	Ľ	F	L	v	A	T	A	T	G	v	H
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	TGG	GTG	171 CGC														TTA	
	W	v	R	Q	A	P	G	K	G	L	E	w	V .,		 E		 L	 P
			225	•		234			243			25 2			261			270
	GGA	AÇT															CTC	
	G	S	N	N	s	R	. Y	N	E	K	F	K	G	R	V	Ţ	v	T
			279			288												324
	AGA 	GAC	ACA	TCC	AÇA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	S	Ţ	N	T	A	Ā	M	E	L	S	s	, L	R	S	E
	GAC	አ ጉ	333	רשכ		342			351						359			378
					TAT	111	1G1	GCA	AGA	100	TAC	GAC	1.1.1.	GCC	7GG	777	GCT	TAC
	D	T	A	V	Ā	Y	С	A	R	S			F	A	W	F	A	Y
	TGG	GGC	387 CAA	GGG	ACT	396 CTG	GTC	ACA	405 GTC	TCC	TCA	414 GCC	TCC	ACC	423 AAG	GGC	CCA	432
	W	G	Q	G	T	L	V	T	V		S	A	S	T	K	G	P	S
	GTC	TTC	441 CCC			450 CCC								GGC			GCC	
	v	F	P	L	A	p	s	S	K	S	T	s	G	G	 T	 A	 A	
						50,4									531		*	540
	GGC	TGC	CTG	GTC	DAA 	GAC	TAC	TTC	ccc	AAD 	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
	G	C	L	A	K	D	Y	F	P	E	₽	V	T	v	S	W	N	S
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				ACC	AGC		GTG	CAC	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	G	٨	L	T	S	G	v .	Ħ	T	F	P	A	V	L	Q	ន	ຮ	G
	CTC	TAC	603 TCC			612 AGC			621 ACC								ACC	648 CAG
		 Ƴ	 s	 L	 S	 S	 v	v	T	v	 P	 S	 5		 L			
			657		-	666			675			584			693			702



ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSNTKVDKK 738 711 729 747 756 720 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT VEPKSCDKTHTCPPCPAP 765 774 783 792 801 810 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT. 819 828 837 846 855 854 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC PEVTCVVV R 873 882 891 900 909 918 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT E D P E V K F N W Y V D G V E V H 927 935 945 . 954 963 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC R V V AKTKPREEQYN5TY 990 999 1008 1017 981 CTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N G K E Y K C K 1035 1044 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T I S K A K 1107 1116 1125 1098 1089 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG V Y GQPR 1152 1161 1170 1179 1143 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC Q V S T C LVKGFY T K N 1224 1206 1215 1233 1242 1197 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG E W E S N G Q P E N N Y K 1296 1251 1260 1269 1278 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG D C S Y 1323 1332 1341 1305 1314 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG

D K S R W Q Q G N V F S C S V M H

03/04 00 MON 14:46 FAX 01159 552201 ERIC POTTER CLARKSON 1359 1368 1377 1386 1395 GCT CTG CAC AAC CAC TAC ACC CAC AAC ACC CTC TCC CTC TCT CCC GGT AAA CCC ALHNHYTOKSLSPGKG 1413 1422 1431 1440 1449 AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG S G G L K I A A F N I Q T F G E 1467 1476 1485 1494 1503 ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC M S N A T L V S Y I V Q I L S R Y D 1530 1539 1548 1521 1557 ATC CCC CTC CTC CAC GAG GTC AGA GAC AGC CAC CTG ACT CCC CTC CCC AAC CTC IALVQEVRDSHLTAVGKL 1575 1584 1593 1602 1611 1620 CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG L D N L N Q D & D D T Y H Y V. V S E 1629 1638 1647 1656 1665 1574 CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC PLGRNSYKERYLFVYRPD 1683 1692 1701 1710 1719 ^{*} CAG GTG TCT GCG GTG GAC AGC TAC TAC TAC CAT CAT CCC TGC GAG CCC TGC GGG O V S A V D S Y Y Y D D G C E P C G 1737 1746 1755 1764 1773 AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA NDTFNREPAIVRFF5 T 1800 1809 1818 1827 1836 1791 GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA V R E F A I V P L H A A P G D A V 1845 1854 1863 1872 1881 1890 GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC EIDALYDVYLDVQEKWG 1899 1908 1917 1926 1935 TTC CAC CAC CTC ATC TTC ATC GGC GAC TTC AAT GCG QGC TCC ACC TAT GTG AGA EDV MLM GDFNAGC 1953 1962 1971 1980 1989 CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG SSIRLWTSP T F 2007 2016 2025 **ZO34** 2043 ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG

T A

PTHCAY

2079 2088 2070 2061 2097 2106 ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT I V V A G M L L R G A V V P D S A L 2115 2124 2133 2142 2151 2160 CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC PFNFQAAYGLSDQL_QAQAI 2169 2178 2187 2196 2205 AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG E V M L K G G

2223 .CGC AAG GTT TGA 3' R K V *

FIGURE !

(A) pAS38

PAS38.DNA LOCUS 2223 bp 2193 bp DNA 14-AUG-1998 HUMANISED HMFG1 heavy chain fused to human DNAse construct 38 DEFINITION Clone 17.12.1 with silent K to K mutation (1398 A > G)+NLS DEFINITION REFERENCE AUTHORS VERHOEYEN ET AL CONSTRUCTION OF RESHAPED HMFG1 etc TITLE JOURNAL IMMUNOL. (1993):78, 364-370 Human DNAse sequence is modified as a result of oligo assembly COMMENT (mhdnase.dna) The fusion was made using overlapping oligos AS81 and AS82 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) FEATURES Residue 963 is G > T leading to silent mutation in all clones FEATURES In 17.12.1 residue 1398 is A > G (silent K to K mutation) FEATURES SITES Note 510 a BASE COUNT 683 c 618 q 412 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 51 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA CCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGC CTGAAGATCG CAGCCTTCAA CATCCAGACA 1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC 1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG 1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA 1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT 1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC 1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT 1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC 1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT 1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC 1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC 2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT 2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT 2221 TGA

LOMES

11

File: PAS38.DNA

FICURE 11 (B) 1 - 2223 Mode: Normal Codon Table : Universal 36 45 27 5' ATG GGA TGG ACC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC F L Α S C I I L v T A M 99 108 90 72 81 63 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAC CTC AAA AAG CCT CGG GCC TCA SCAEVK PGAS 144 153 117 125 135 GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAC s G ¥ C K A 198 180 189 207 215 171 TCC.GTG CGC CAC GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT Q A P G K G L E WV 270 234 243 252 261 225 GGA AGT AAT AAT TOT AGA TAC AAT GAG AAG TTO AAC CCC CGA GTG ACA GTC ACT SRYNEKFKG RVTVT 279 288 297 306 324 315 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG A Y M Ε LSSLRSE 333 342 351 360 369 378 GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC YYCARSYDF 396 387 405 414 423 432 TGG GGC CAA GGC ACT CTC GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG L V T 5 v 5 A 5 441 450 459 477 468 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GCC ACA CCC CCC CTG SKSTSGG T A 495 504 513 522 531 540 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GCLVKDY F P E P V T V SWNS 549 558 567 576 585 594 GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA GALTSGVH T A V L F Q S 603 612 621 630 639 CTC TAC TCC CTC ACC ACC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG S T S S 657 666 675 684 693 702

- 1 -

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA K V N P S K V N H 756 747 738 729 720 711 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT H K Œ 810 783 792 801 774 765 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC PSVFLFPPKP 337 855 819 828 845 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC E 873 891 900 909 918 882 CAA CAC CCT GAG CTC AAC TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT E D P E V F ${f N}$ Y K W 927 936 945 954 963 972 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC AKTK E E QYNS R 981 990 999 1008 1017 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG H D L N 1035 1044 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA A L P APIEK ISKAK 1089 1098 1107 1116 1125 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG P Q V Y 1143 1152 1161 1170 1179 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC TKNQVSL TCLVKGFYPSD 1197 1206 1215 1224 1233 1242 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG WESNGQPENNYKTT IAVE 1251 1260 1259 1278 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVL D S D G S F F L Y S K L 1305 1314 1323 1332 1341 1350 CAC AAC ACC ACC TCC CAC CAG GGG AAC CTC TTC TCA TCC TCC GTG ATG CAT GAG D K S R W Q Q G N V

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1359 1368 1377 1386 1395 1404 . CCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC H Y T Q K S L S P K G S 1422 1431 1440 1449 1458 1413 GGC GGG CTG AAC ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG G-G L K I A A F N I Q T F G E T K M 1467 1476 1485 1494 1503 1512 TCC ANT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTC ACC CGC TAC GAC ATC S N A T L V S Y I V Q I L S R Y D I 1521 1539 1548 1557 1530 GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG ALVQEVRDSHLTAVCKLL 1575 1584 1593 1602 1611 1620 GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA DNLNQDAPDTYHYVV5EP 1629 1638 1647 1656 1665 CTG GGA CGG AAC AGC TAT AAC CAC CCC TAC CTC TTC GTG TAC AGG CCT GAC CAG LGRNSYKERYLFVYRPDQ 1683 1692 1701 1710 1719 GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC DSAAADDC P C 1737 1746 1755 1764 1773 GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG DTFNREPAIVRFFSRFTE 1791 1800 1809 1818 1827 GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC V R E F A I V P L H A A P G D A V A 1845 1854 1863 1872 1881 GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG EIDALYDVYLDVQEKWGL 1899 1908 1917 1926 1935 1944 GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC EDVMLMGDFNACCSYVED 1962 1971 1953 1980 1989 1998 TCC 'CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC S Q W S S I R L W T S P T F Q W L I 2007 2016 2025 2034 2043 2052 CCC GAC AGC GCT GAC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC

PDSADTTATPTHCAYDR1

2079 2088. 2097 2061 2070 GTG GTT GCA GGG ATG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC V V A G M L L R G A V V P D S A L P 2124 2133 2142 · 2151 2115 TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT F M F Q A A Y G L S D Q L A Q A I S

2187 2169 2196 2205 2178 CAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC DHYPVE-VMLKGGGPKKKR

2223 AAG GTT TGA 3' K V *

FIGURE 12

pAS39

2220 bp 2190 bp 14-AUG-1998 DNA PAS39.DNA LOCUS DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse - construct 39 Clone 18.24.1 with residue 1392 T > C +NLS DEFINITION REFERENCE

VERHOEYEN ET AL AUTHORS

CONSTRUCTION OF RESHAPED HMFG1 etc TITLE

IMMUNOL. (1993):78, 364-370 JOURNAL

Human DNAse sequence is modified as a result of oligo assembly COMMENT

(mhdnase-dna)

The fusion was made using overlapping oligos AS83 and AS84 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) FEATURES Residue 963 is G > T leading to silent mutation in all clones FEATURES Residue 1392 T > C silent S to S mutation FEATURES

Note SITES

684 c 617 g 411 t BASE COUNT 508 a

ORIGIN

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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
  61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CCCCGGGGAG CGCCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
1741 GAGCCAGCCA TIGICAGGIT CITCICCGG TICACAGAGG TCAGGGAGIT TGCCATIGIT
1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT
2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC
2161 CACTATCCAG TGGAGGTGAT GCTGAAGGGG GGCGGACCCA AAAAGAAGCG CAAGGTTTGA
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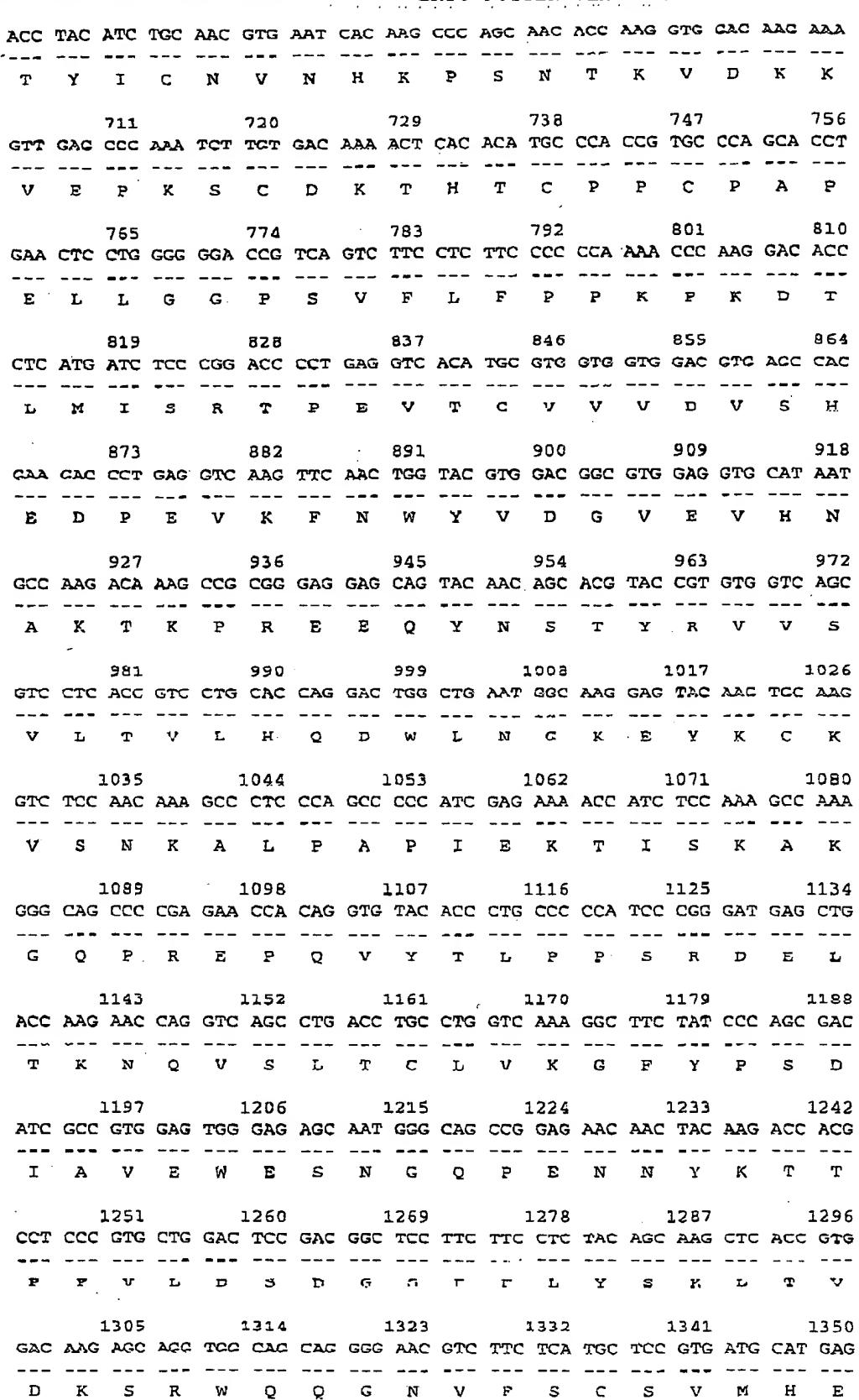
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Codon Table: Universal

FIGURE 12(B)

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V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
		495			504			513			522			531			540
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		657			666			675			684			693			702

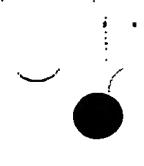
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1368 1377 1986 1395 1404 1359 ·GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCC CCC GGG AGC CCC A L H N H Y T Q K S L S L S P G S G 1422 1431 1440 1449 1458 1413 GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG TCC G L K I A A F N I Q T F G E T K M S 1476 1485 1494 1503 1512 1467 AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC GCC NATLVSYIVQILSRYDIA 1539 1548 1557 1521 1530 CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG CCC AAC CTG CAC LVQEVRDSHLTAVGKLLD 1611 1575 1584 1593 1602 AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA CTG NLNQDAPDTYHYVVSEPL 1538 1647 1656 1665 1529 GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG GTG GRNSYKERYLFVYRPDQV 1701 1710 1719 1683 1592 TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT CCC TGC GAG CCC TGC GGC AAC GAC SAV D S Y Y D D G C E P C G N D 1737 1746 1755 1764 1773 1782 ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG GTC T F N R E P A I V R F F S R F T E V 1800 1809 1818 1827 1836 1791 AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC GAG 1845 1854 1863 1872 1881 1890 ATC CAC CCT CTC TAT GAC CTC TAC CTC CAT GTC CAA GAG AAA TGG GGC TTG GAG I D A L Y D V Y L D V Q E K W G L E 1899 1908 1917 1926 1935 GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC TCC D V M L M G D F N A G C S Y V R P S 1953 1962 1971 1980 1989 CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC CCC Q W S S I R L W T S P T F QWLJP 2007 2016 2025 2034 2043 GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC GTG DSADTTATPTHCAYDRIV

- 3 -



2061 2070 2079 2088 2097 2106 GTT GCA GGG ATG CTC CGA GGG GCC GTT GTT CCC GAC TCG GCT CTT CCC TTT ·v a c m l l R G A V V P D S A L P F 2133 2142 2151 2160 2115 2124 AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC AGT GAC N F Q A A Y G L S D Q L A Q A I S D 2169 2178 2187 2196 2205 2214 CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG CGC AAG V M L K G G G P K K R K

GTT TGA 3'

06-MAR-1995

PRI .

FIGURE 13

(A) pAS101

mRNA 1548 bp PAS101.DNA LOCUS DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS101) ACCESSION NID KEYWORDS DNase I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASEl_dna) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL MEDLINE 91067672 343 a 467 c 430 g 308 t BASE COUNT ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC 781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT 841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG 901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC 961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG 1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG 1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC 1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC 1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG 1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC 1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA 1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT 1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG 1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA

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To-THE PATENT OFFICE

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File: PAS101.DNA
Range: 1 - 1548
Codon Table: Universal

Mode : Normal

FICURE 13(8)

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GAC	ACA			TAT			GCA	AGA								GCT	
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·		927			936			945			954			963			972
CTG L	GAC D	927 AAC N 981	CTC L	AAT N	936 CAG Q 990	GAC	GCA A	945 CCA P 999	GAC D	ACC T	954 TAT Y	CAC 	TAC	963 GTG V 1017	GTC V	AGT S	972 GAG E
CTG L	GAC D	927 AAC N 981	CTC L	AAT N	936 CAG Q 990	GAC	GCA A	945 CCA P 999	GAC D	ACC T	954 TAT Y	CAC 	TAC	963 GTG V 1017	GTC V	AGT S	972 GAG E
CTG L	GAC D	927 AAC N 981	CTC L	AAT N	936 CAG Q 990	GAC	GCA A	945 CCA P 999	GAC D	ACC T	954 TAT Y	CAC 	TAC	963 GTG V 1017	GTC V	AGT S	972 GAG E
CTG L CCA	GAC D CTC	927 AAC N 981 CCA	CTC L CCG	AAT N AAC N	936 CAG Q 990 AGC	GAC D TAT 	GCA A AAG	945 CCA P 999 GAG	GAC D CGC	TAC	954 TAT Y 1008 CTG	CAC H TTC	TAC Y GTG	963 GTG V 1017 TAC	GTC V AGG	AGT S CCT 	972 GAG E 1026 GAC
CTG L CCA	GAC D CTC	927 AAC N 981 CCA C	CTC L CCG	AAT N AAC N	936 CAG Q 990 AGC S	GAC D TAT 	GCA A AAG	945 CCA P 999 GAG E	GAC D CGC 	ACC T T	954 TAT Y 1008 CTG L	CAC H TTC	TAC Y GTG	963 GTG V 1017 TAC Y	GTC V AGG	AGT S CCT 	972 GAG E 1026 GAC D
CTG L CCA	GAC D CTC	927 AAC N 981 CCA C	CTC L CCG	AAT N AAC N	936 CAG Q 990 AGC S	GAC D TAT 	GCA A AAG	945 CCA P 999 GAG E	GAC D CGC 	ACC T T	954 TAT Y 1008 CTG L	CAC H TTC	TAC Y GTG	963 GTG V 1017 TAC Y	GTC V AGG	AGT S CCT 	972 GAG E 1026 GAC D
CTG L CCA D	GAC D CTC L GTG V	927 AAC N 981 CCA C	CTC L CCC R	AAT N AAC N GTG V	936 CAG Q 990 AGC S	GAC TAT Y AGC	GCA A AAG K TAC Y	945 CCA P 999 GAG E	GAC D CGC R TAC	ACC T T TAC Y GAT D	954 TAT Y 1008 CTG L 1062 GAT	CAC H TTC F	TAC Y GTG V TGC	963 GTG V 1017 TAC Y 1071 GAG	GTC V AGG R CCC	AGT S CCT P	972 GAG E 1026 GAC D
CTG L CCA P CAG	GAC D CTC L GTG	927 AAC N 981 CCA G 1035 TCT S	CTC L CCC R	AAT N AAC N GTG V	936 CAG Q 990 AGC S 1044 GAC D	GAC TAT Y AGC	GCA A AAG X TAC	945 CCA P 999 GAG E 1053 TAC Y	GAC D CGC R TAC	ACC TT TAC Y GAT D	954 TAT Y 1008 CTG L 1062 GAT D	CAC H TTC F GGC G	TAC Y GTG V TGC	963 GTG V 1017 TAC Y 1071 GAG E	GTC V AGG R CCC	AGT S CCT P	972 GAG E 1026 GAC D 1080 GGG G
CTG L CCA P CAG	GAC D CTC L GTG V	927 AAC N 981 CCA G 1035 TCT S	CTC L CCC R	AAT N AAC N GTG V	936 CAG Q 990 AGC S 1044 GAC D	GAC TAT Y AGC	GCA A AAG TAC Y CCA	945 CCA P 999 GAG E 1053 TAC Y	GAC D CGC R TAC	ACC T T TAC Y GAT D	954 TAT Y 1008 CTG L 1062 GAT D	CAC H TTC F GGC G	TAC Y GTG V TGC	963 GTG V 1017 TAC Y 1071 GAG E	GTC V AGG R CCC	AGT S CCT P	972 GAG E 1026 GAC D 1080 GGG G
CTG L CCA D CAG	GAC D GTG V GAC D	927 AAC N 981 CCA C 1035 TCT S 1089 ACC T	CTC L CCG R GCG A TTC	AAT N AAC N AAC N	936 CAG Q 990 AGC S 1044 GAC D 1098 CGA R	GAC D TAT Y AGC S	GCA A AAG TAC Y CCA P	945 CCA P 999 GAG E 1053 TAC Y	GAC D CGC R TAC Y ATT	TAC TAC Y GAT D GTC V	954 TAT Y 1008 CTG L 1062 GAT D	CAC H TTC F	TAC Y GTG Y TGC F	963 GTG V 1017 TAC Y 1071 GAG E 1125 TCC	GTC V AGG R CCC R	AGT S CCT P TGC C	972 GAG E 1026 GAC D 1080 GGG G
CTG L CCA D CAG O	GAC D GTG V GAC D	927 AAC N 981 CCA C 1035 TCT S 1089 ACC T	CTC L CCC R GCG A TTC	AAT N AAC N AAC N	936 CAG Q 990 AGC S 1044 GAC D 1098 CGA R	GAC D TAT Y AGC S	GCA A AAG TAC Y CCA P	945 CCA P 999 GAG E 1053 TAC Y 1107 GCC A	GAC D CGC R TAC Y ATT	TAC TAC Y GAT D	954 TAT Y 1008 CTG L 1062 GAT D	CAC H TTC F TTC F	TAC Y GTG Y TGC F	963 GTG V 1017 TAC Y 1071 GAG E 1125 TCC S	GTC V AGG R CCC R	AGT S CCT P TGC C	972 GAG E 1026 GAC D 1080 GGG G
CTG L CCA D CAG N GAG	GAC TO GTG V GAC D GTC	927 AAC N 981 CCA S 1035 TCT S 1089 ACC T 1143 AGG	CTC L CCC R GCG A TTC F	AAT N AAC N AAC N TTT	936 CAG Q 990 AGC S 1044 GAC D 1098 CGA R 1152 GCC	GAC D TAT S GAG S ATT	GCA A AAG X TAC Y CCA P	945 CCA P 999 GAG E 1053 TAC Y 1107 GCC A	GAC D CGC X TAC Y ATT I CTG	ACC T TAC Y GAT D CAT	954 TAT Y 1008 CTG L 1062 GAT R 1170 GCG	CAC H TTC F GCC	TAC Y GTG V TCC F CCG	963 GTG V 1017 TAC Y 1071 GAG S 1125 TCC S	GTC V AGG R CCC R CGG	AGT S CCT P TGC C	972 GAG E 1026 GAC D 1080 GGG G 1134 ACA T
CTG L CCA D CAG O	GAC D CTC L GTG V GAC V	927 AAC N 981 CCA C 1035 TCT S 1089 ACC T	CTC L CCC R GCG A TTC F	AAT N AAC N TTT F	936 CAG Q 990 AGC S 1044 GAC D 1098 CGA R	GAC D TAT Y AGC S ATT I	GCA A AAG Y CCA P GTT V	945 CCA P 999 GAG E 1053 TAC Y 1107 GCC A	GAC D CGC R TAC Y ATT I CTG L	ACC T T TAC Y GAT D CAT H	954 TAT Y 1008 CTG L 1062 GAT D	CAC H TTC F GCC A	TAC Y GTG Y TCC F CCG	963 GTG V 1017 TAC Y 1071 GAG E 1125 TCC S	GTC V AGG R CCC R CGG	AGT S CCT P TGC C TTC F	972 GAG E 1026 GAC D 1080 GGG G

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GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG CCC A E I D A L Y D V Y L D V Q É K W G 1251 1260 1269 1278 1287 1296 TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA L E D V M L M G D F N A G C S Y V R 1305 1314 1323 1332 1341 1350 CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG PSQWSSIRLWTSPTFQWL 1359 1369 1377 1386 1395 1404 ATC CCC CAC AGC GCT CAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG I P D S A D T T A T P T H C A Y D R 1413 1422 1431 1440 1449 1458 ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCC CCT CTT I V V A G M L L R G A V V P D S A L 1467 1476 1485 1494 1503 1512 CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC PFNFQAAYGLSDQLAQAI 1521 1530 1539 1548 AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

-3-

S D H Y P V E V M L K *



FIGURE 14

(A) pAS102

PAS102.DNA 1566 bp mRNA PRI 06-MAR-1995 Locus Humanised HMFG1 Fab'2 fused to human DNase I (pAS102) DEFINITION ACCESSION ` NID KEYWORDS DNasa I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) (see Figure 2) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL

MEDLINE 91067672

BASE COUNT 345 a 469 c 440 g 312 t

ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC 781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC 841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG 901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA 961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC 1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT 1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC 1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC 1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC 1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC 1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC 1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG 1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT 1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG 1561 AAGTGA

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' File : PAS102.DNA Range : 1 -Range: 1 - 1566 Codon Table: Universal

Mode : Normal

FIGURE 14 (B)

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V	ĸ	V	s	С	ĸ	·A	s	G	Y	T	F	S	A	Y	M	I	E
		171			180			189			198			207			216
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
W	v	R	Q	A	P	G	К	G	r	E	W	V	G	E	I	L	P
		225			234			243			252			261			270
GGA	AGT			TCT			AAT			TTC			CGA	GTG	AÇA	GTC	ACT
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		220			288			207			306			315			324
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GAÇ	ACA	333 333 ،		TAT	342 TAC		' ÇCA	351 aca		מת :	360 Cac		, GCC	369 TÇC		CCT	378 TAC
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TIC	: cc	387		ACT	396 CTC		. ACA	405 GTC		TCA	414 3 GCC		: ACC	423 : AAG		CCA	432 TCG
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		495			504			513			523			533			540
GGC	TGO	CTC	GTO	AAC	GAC	TAC	TTC		GAA	A CCC	G GTO	ACC	GT	TCC	3 TG(3 AAC	TCA
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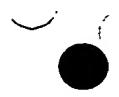
612 621 630 639 648 603 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG TVPSSSLGTQ s v v S L S 657 · 675 693 702 684 **ნ** ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAC CTG GAC AAC AAA T Y I C N V N H K P 5 N T K V D K K 720 729 738 747 711 GTT GAG CCC AAA TCT TCT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG TCCVECPP EPKSCDKTH 774 783 792 801 765 TGC CCA GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG G S G C L K I A A F N I Q E 855 837 846 819 **8**28 ACA TIT GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG T K M S N A T L V IVO 891 900 909 918 873 ATC CTG AGC CGC TAC GAC ATC GCC CTG CTC CAG GAG GTC ACA CAC AGC CAC CTG LSRYDIALVQBV 945 936 954 963 972 927 ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG.GAC GCA CCA GAC ACC TAT A P TAVGKLLDNLNQD 1008 1017 999 981 990 CAC TAC GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG KERYL Z X R N 1062 1071 1035 1044 1053 TTC GTG TAC AGG CCT GAC CAG GTG TCT GCG GTG GAC AGC TAC TAC GAT GAT V S A V D Y Y Y S D Ω. 1098 1107 1116 1125 1134 1089 CGC TGC GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG CCNDTFNREPAIVR 1179 116**1** 1170 1152 1143 TTC TTC TCC CGG TTC ACA CAC CTC AGG GAG TTT CCC ATT GTT CCC CTG CAT GCG FSRFTEVREFA 1197 1206 1215 1224 1233 1242

-2-

GCC CCG GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT A P G D A V A E I D A L 'Y D V Y L D 1251 1260 1269 1278 1287 1296 GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG V Q E K W G L E D V M L M G D F N A 1305 1314 1323 1332 1341 1350 GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC G C S Y V R P S Q W S S I R L W T S 1368 1377 1386 1395 1404 1359 CCC ACC TTC CAG TGG CTC ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG PTFQWLIPDSADTTATPT 1413 1422 1431 1440 1449 1458 CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTC CGA GGG GCC GTT H C A Y D R I V V A G M L L R G A V 1467 1476 1485 1494 1503 1512 GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC V P D S A L P F N F Q A A Y G L S D 1521 1530 1539 1548 1557 1565 CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3' Q L A Q A I S D H Y P V E V M L K

PRI

06-MAR-1995



LOCUS

FIGURE 15

PAS103.DNA

pAS103

mRNA

1560 bp

DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS103) ACCESSION NID DNase I. KEYWORDS DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL 91067672 MEDLINE 468 c 436 g 312 t 344 a BASE COUNT ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTICA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTCCTCAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AACTTCAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG 781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA 901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG 1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC 1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG 1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA 1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG 1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG 1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC 1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC 1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG 1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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File: PAS103.DNA Range :

Codon Table : Universal

1 - 1560 Mode: Normal

FIGURE 15 (B)

27 36 45 18 5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC IILFL V A C 90 72 81 63 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA VQSGAEVK 153 144 135 126 117 GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG A Y W V K V S C K A S G Y F 207 180 198 189 171 TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT WVRQAPGKGLEWVGEI 243 261 270 234 252 225 GGA AGT AAT AAT TOT AGA TAC AAT GAG AAG TTO AAG GGO CGA GTG ACA GTO ACT G S N N S R Y N E K F K G 324 315 297 306 288 279 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG AYMELS 369 378 360 342 351 333 GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT CCC TGG TTT GCT TAC Y D S X Y A R D TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG WGQGTLVTVSSASTKGP 468 477 485 450 459 441 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG S S K S T S V F P L A P G G TAAL 513 522 531 540 495 504 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA G C L V K D Y F P E P V T · V S W N S 567 576 594 558 585 549 GGC GCC CTG ACC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA PAVLQSSC SCVH - 1 -



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GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA G D A V A E I D A L Y D V Y L D V Q 1251 1260 1269 1278 1287 1296 GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC EKWGLEDVMLMGDFNAGC 1314 1323 1332 1341 1350 1305 AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC S Y V R P S Q W S S I R L W T S P T 1368 1377 1386 1395 1404 1359 TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT FQWLIPDSADTTATPTHC 1422 1431 1440 1449 1413 GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CCA CCG GCC GTT GTT CCC AYDRIVVAGMLLRGAVVP 1476 1485 1494 1503 1512 1467 GAC TOG GOT CTT CCC TTT AAC TTC CAG GOT GCC TAT GGC CTG AGT GAC CAA CTG DSALPFNFQAAYGLSDQL 1521 1530 1539 1548 1557 GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3'

A O A I S D H Y P V E V M L K *

FIGURE 16

(A) pAS104

mRNA PRI 06-MAR-1995 PAS104 DNA 1560 bp LOCUS DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS104) Position 924 G to A by ggg to gag Linker GR instead of GG (position 777) ACCESSION NID KEYWORDS DNase I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) Homo sapiens ORGANISM Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL MEDLINE 91067672 468 c 434 g 312 t BASE COUNT 346 a ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TITCCCTGGT TIGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 491 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCACCGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 501 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTCC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCAGGCTG 781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA 901 GACAGCCACC TGACTGCCGT GGAGAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG 1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC 1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG 1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA 1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG 1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG 1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC 1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC 1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG 1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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File: PAS104.DNA
Range: 1 - 1560
Codon Table: Universal

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Mode : Normal

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			441	•		450			459			468			477			486
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AGC	CGC	TAC	GAC	ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC
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GTG	GTC	981			990			999	-		1008		:	_	-	:	1026
GTG V	GTC V	981			990			999	-		1008		:	1017	-	:	1026
	 V	981 AGT	GAG E	CCA P	990 CTG	GGA	CGG R	999 AAC N	AGC	ТАТ Y	1008 AAG	GAG E	CGC R	1017 TAC	CTG L	TTC F	LOZ6 GTG
V	v	981 AGT \$	GAG E	CCA P	990 CTG L	GGA G	CGG R	999 AAC N 1053	AGC S	TAT Y	1008 AAG K 1062	GAG E	CGC R	1017 TAC Y	CTG L	TTC	1026 GTG V
V	V AGG	981 AGT S 1035 CCT	GAG E	CCA P	990 CTG L	GGA G	CGG R	999 AAC N 1053	AGC S	TAT Y	1008 AAG K 1062	GAG E	CGC R	1017 TAC Y	CTG L	TTC	1026 GTG V
V TAC	V AGG	981 AGT S 1035 CCT 	GAG E GAC	CAG	990 CTG L 1044 CTC V	GGA G TCT	CGG R CCG	999 AAC N 1053 CTG V	AGC S GAC	TAT Y AGC	1008 AAG K 1062 TAC 	GAG E TAC	CGC R TAC	1017 TAC Y 1071 GAT	CTG L GAT	TTC F GGC	1026 GTG V 1080 TGC
V TAC	V AGG	981 AGT S 1035 CCT P	GAG E GAC	CCA P CAG	990 CTG L 1044 CTC V	GGA G TCT	CGG R CCG	999 AAC N 1053 GTG V	AGC S GAC	TAT Y AGC	1008 AAG K 1062 TAC 	GAG E TAC	CGC R TAC	1017 TAC Y 1071 GAT	CTG L GAT	TTC F GGC	1026 GTG V 1080 TGC C
V TAC Y GAG	AGG R CCC	981 AGT \$ 1035 CCT P 1089 TGC	GAG E GAC D GGG	CCA P CAG Q	990 CTG L 1044 CTC V	GGA G TCT S	CCC A	999 AAC N 1053 CTG V 1107 AAC	AGC S GAC D CGA	TAT Y AGC S	1008 AAG K 1062 TAC Y	GAG E TAC Y GCC	CGC R TAC Y	1017 TAC Y 1071 GAT D	CTG L GAT D	TTC	1026 GTG V 1080 TGC C
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V TAC Y GAG	AGG	981 AGT S 1035 CCT P 1089 TGC C	GAG E GAC TD	CAG P CAG Q AAC	990 CTG L 1044 CTC V 1098 GAC D	GGA G TCT S	CGG R CCC A	999 AAC N 1053 GTG V 1107 AAC N	AGC S GAC D CGA R	TAT Y AGC S GAG	1008 AAG K 1062 TAC Y 1116 CCA 	GAG E TAC Y GCC A	CGC R TAC Y	1017 TAC Y 1071 GAT D 1125 GTC V	CTG L GAT D	TTC F GGC TTC	1026 GTG V 1080 TGC C 1134 TTC
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- 2 -

GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA G D A V A E I D A L Y D V Y L D V Q 1260 1269 1278 1287 1296 1251 GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC E K W G L E D V M L M G D F N A G C 1305 1314 1323 1332 1341 1350 AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC S Y V R P S O W S S I R L W T S P T 1359 1368 1377 1386 1395 1404 TTC CAC TCC CTG ATC CCC CAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT F Q W L I P D S A D T T A T P T H C 1413 1422 1431 1440 1449 GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC AYDRIVVAGMLLRGAVVP 1476 1485 1494 1503 1512 1467 GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG D S A L P F N F Q A A Y G L S D Q L 1521 . 1530 1539 1548 1557 GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3' AQAISDHYPVEVMLK*

PAS105.DNA

PRI

06-MAR-1995



LOCUS

11

Received

pAS105

1578 bp

mRNA

DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS105) ACCESSION NID KEYWORDS DNase I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASEl.dna) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL 91067672 MEDLINE 473 c 442 g 310 t 353 a BASE COUNT ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 561 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC 781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT 841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG 901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC 961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG 1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG 1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC 1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC 1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG 1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC 1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA 1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT 1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG 1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA 1561 AAGAAGCGCA AGGTTTGA

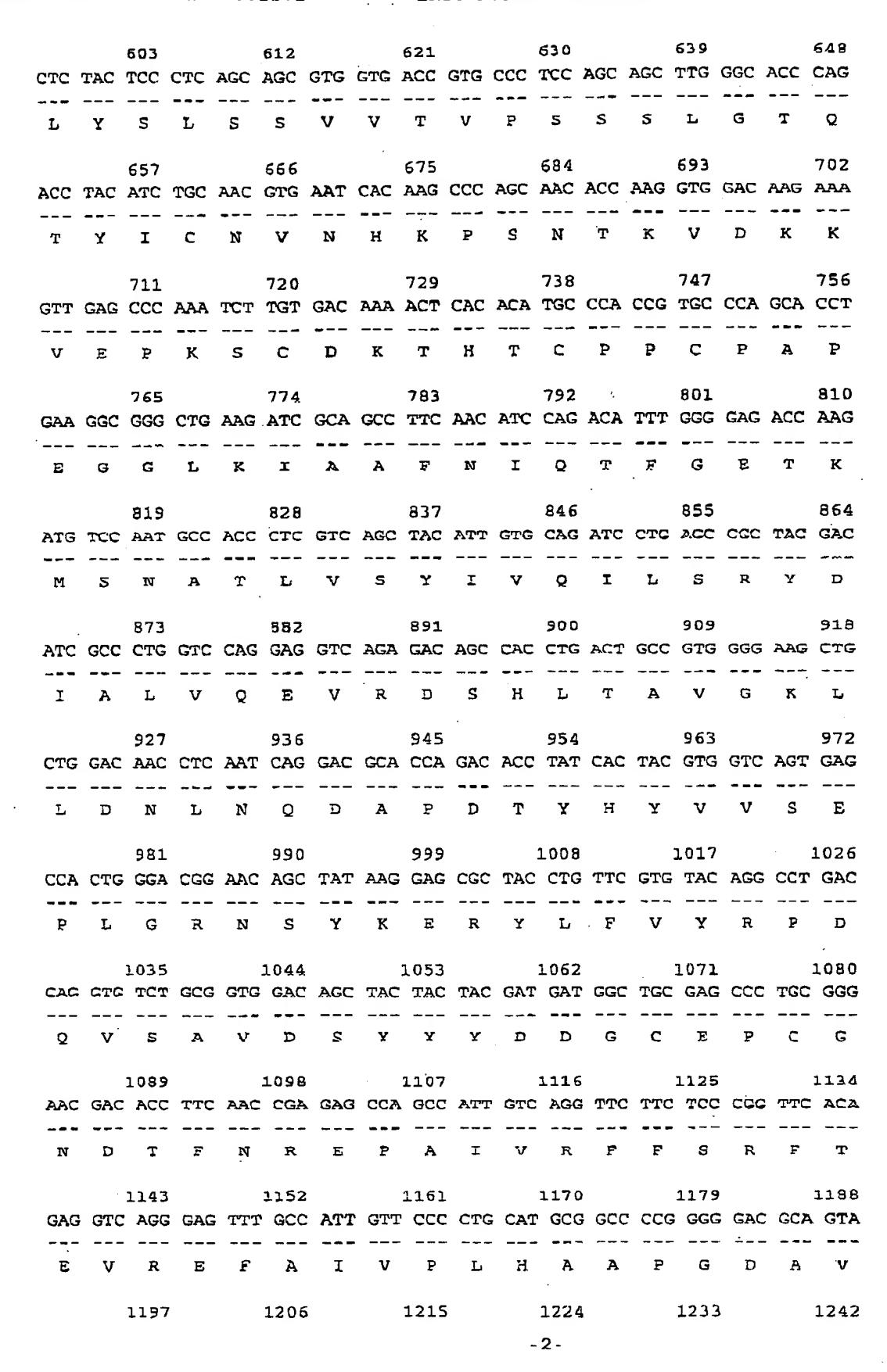
File: PAS105.DNA 1 -Range: Codon Table : Universal

5'

1578 Mode : Normal

FIGURE 17 (B)

45 54 27 36 18 9 ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC V A F L I I L 108 99 90~ 81 72 63 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA OSGAEVK た V 153 144 162 135 126 117 GTG AAG GTG TCC TCC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG **P.** S K 216 207 198 189 180 171 TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT E W V G L G K WVRQAP 261 243 252 225 234 GGA AGT AAT ACT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT SRYNEKFKGRV 324 29**7** 306 315 279 . 288 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG TNTAYMELS S L 369 378 360 351 333 342 GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC A Y V Y Y C A R S Y D D 414 432 396 405 423 387 TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA CCC TCC ACC AAG GGC CCA TCG s a 468 477 486 459 450 441 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG V F P L A P S S K S T S G G T A A L 513 522 531 540 504 495 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA G C L V K D Y F P E P V T V S W N 567 576 585 594 558 549 GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA G A L T S G V H T F P A V L Q S S -] -



GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAC AAA TCG GGC A E I D A L Y D V Y L D V Q E K W G 1251 1260 1269 1278 1287 1296 TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA L E D V M L M G D F N A G C S Y V R 1305 1314 1323 1332 1341 1350 CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG PSQWSSIRLWTSPTFQWL 1359 1368 1377 1386 1395 1404 ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG I P D S A D T T A T P T H C A Y D R 1413 1422 1431 1440 1449 1458 ATC GTG GTT GCA CCG ATG CTG CTC CGA CCC GCC GTT GTT CCC GAC TCG GCT CTT IVVAGMLLRGAVVPDSAL 1467 1476 1485 1494 1503 1512 CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA GCC ATC PFNFQAAYGLSDQLAQAI 1521 1530 1539 1548 1557 1566 AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA CCC AAA AAG AAG SDHYPVEVMLKGGGPKKK 1575 CGC AAG GTT TGA 3'

R K V *

LOCUS

PRI

06-MAR-1995

FIGURE 18

PAS106.DNA

/A) pAS106

mRNA

1596 bp

DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS106) ACCESSION NID KEYWORDS DNase I. DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) ORGANISM Homo sapiens Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL MEDLINE 91067672 355 a 475 c 452 g 314 t BASE COUNT ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AAUGTGAATU ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC 781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC 841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG 901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA 961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC 1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT 1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC 1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC 1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC 1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC 1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC 1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG 1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT 1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG 1561 AAGGGGGGG GACCCAAAAA GAAGCGCAAG GTTTGA

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'File: PAS106.DNA
Range: 1 - 1596
Codon Table: Universal

1 - 1596 Mode: Normal

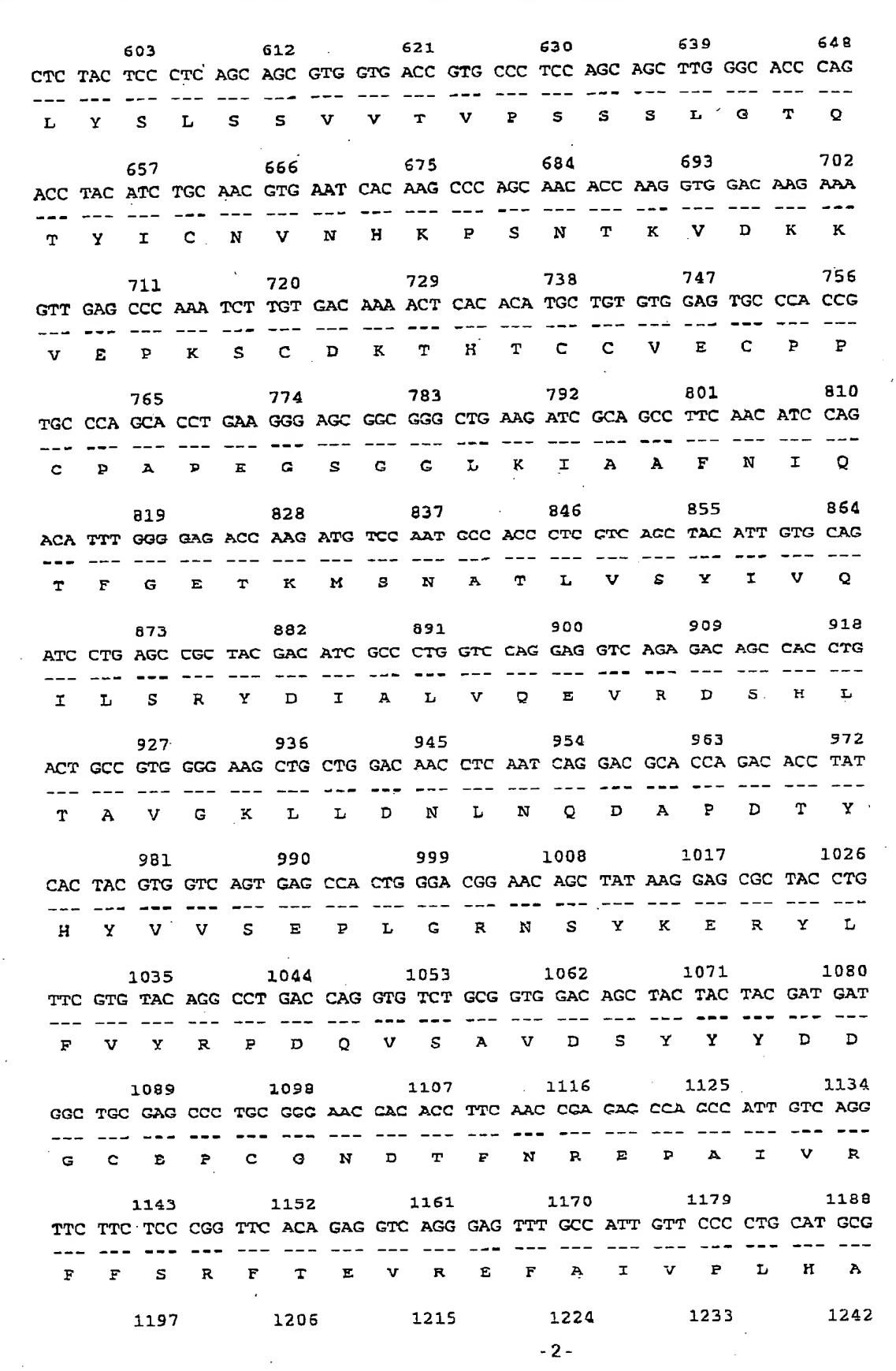
FIGURE 18 K)

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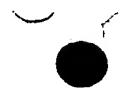


FIGURE 19

PAS107 DNA

(k) pAS107

mRNA

1590 bp

LOCUS DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40 NLS(pAS107) ACCESSION NID DNase I. KEYWORDS DNase I sequence is from assembled oligos (thus modified c/f SOURCE MHDNASE1.dna) Homo sapiens ORGANISM Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata; Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo. Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L. AUTHORS Recombinant human DNase I reduces the viscosity of cystic fibrosis TITLE sputum Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990) JOURNAL 91067672 MEDLINE 448 g 474 c 314 t BASE COUNT 354 a ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG 781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC 841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA 901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC 961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG 1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC 1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG 1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA 1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG 1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG 1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC 1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC 1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG 1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG 1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

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Range: 1 - 1590
Codon Table: Universal

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Mode : Normal

FIGURE 19 (E)

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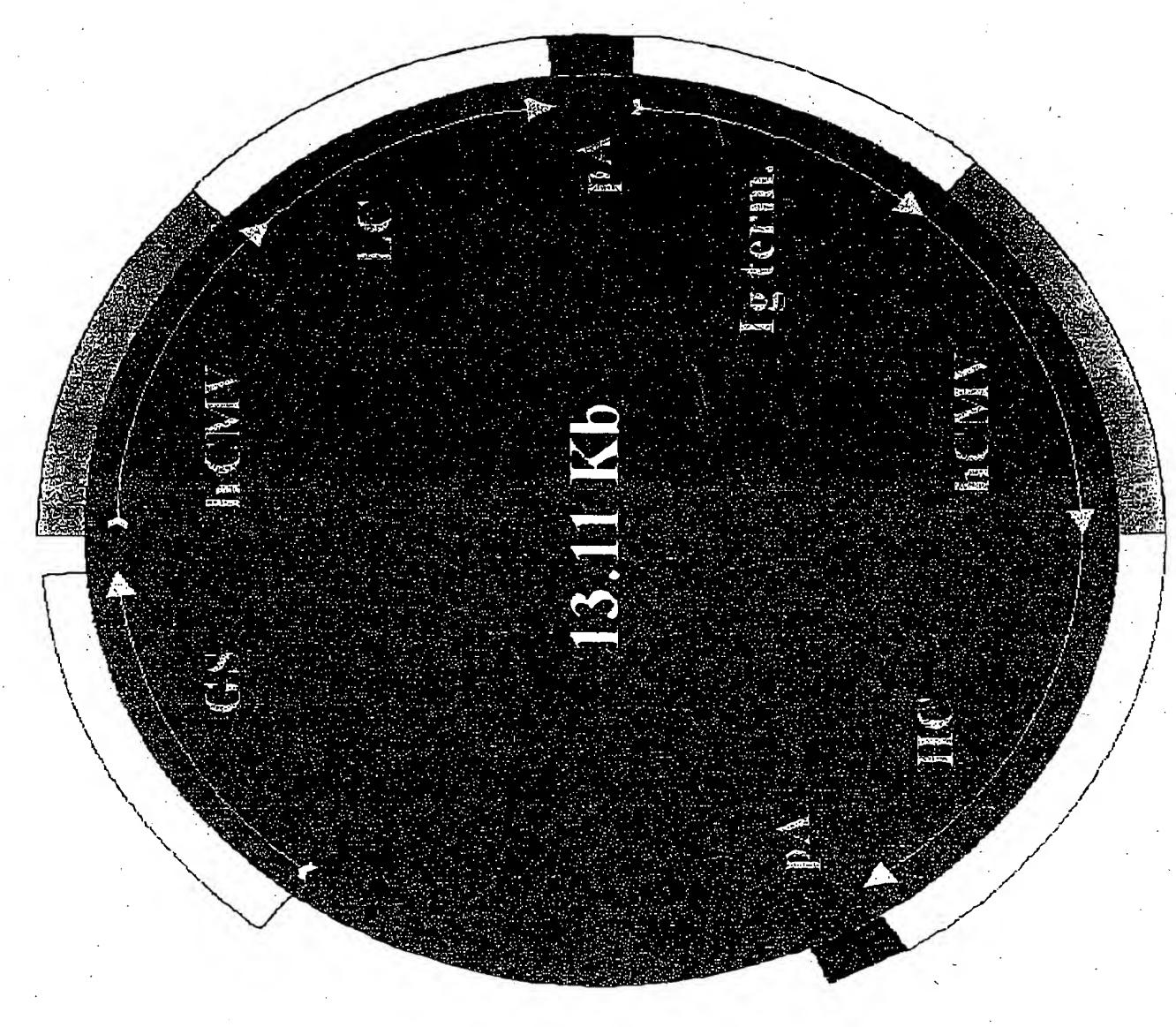
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GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT CTC CAA G D A V A E I D A L Y D V Y L D V Q 1260 1269 1278 1287 1296 1251 GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC E K W G L E D V M L M G D F N A G C 1305 1314 1323 1332 1341 1350 AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC SYVRPSQ.WSSIRLWTSPT 1359 1368 1377 1386 1395 1404 TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT Q W L I P D S A D T T A T P T H C 1413 1422 1431 1440 1449 1458 GCC TAT GAC AGG ATC CTC GTT GCA GGG ATC CTC CTC CGA GGG GCC GTT GTT CCC AYDRIVVAGMLLRCA 1467 1476 1485 1494 1503 1512 GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG DSALPFNFQAAY G L S **1521 1530 1539 1548 1557 1566** GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG GGC GGA A Q A I S D H Y P V E V M L K G G G 1575 1584 CCC AAA AAG AAG CGC AAG GTT TGA 3'

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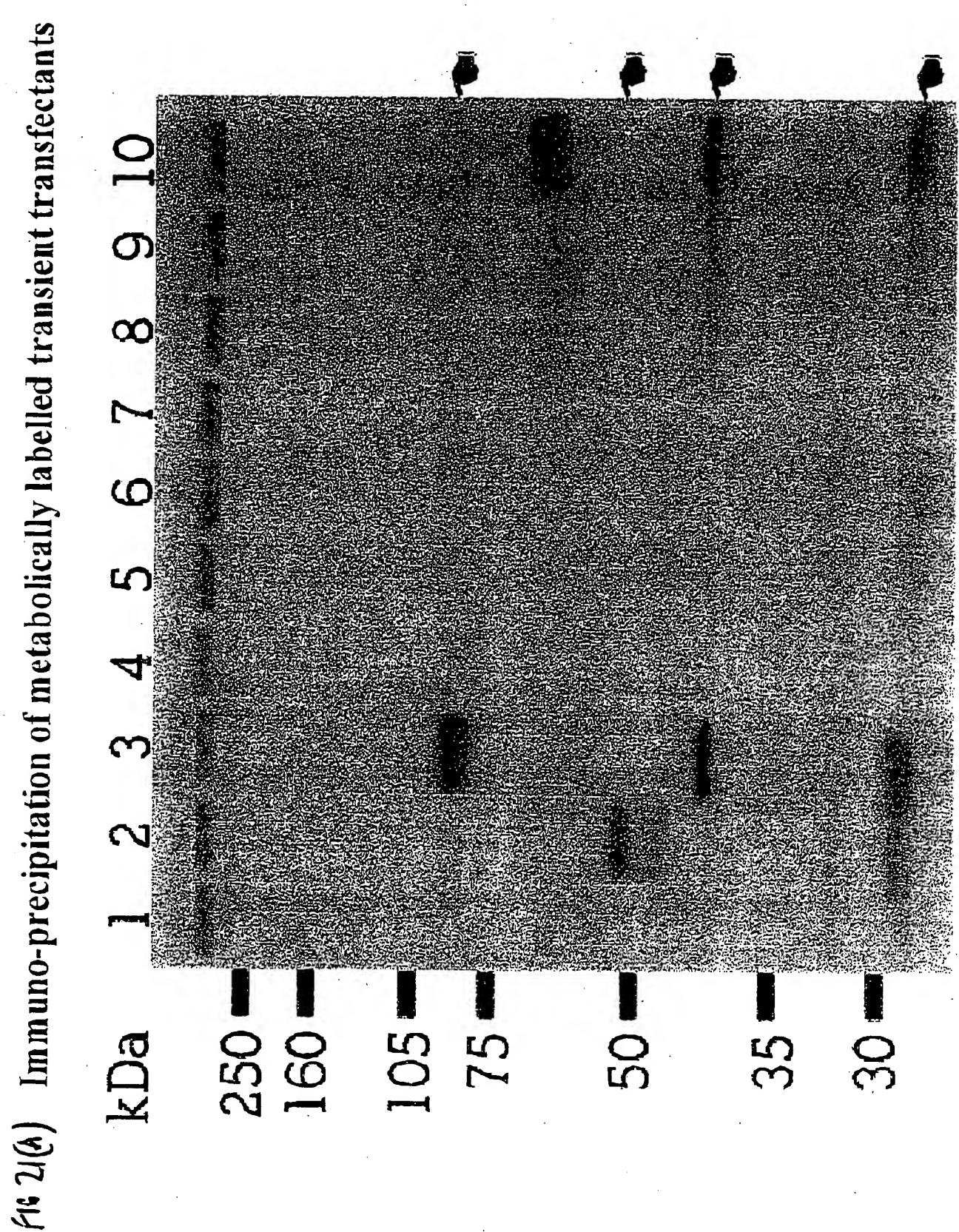
of humanised HMFG1-DNase constructs Mammalian expression



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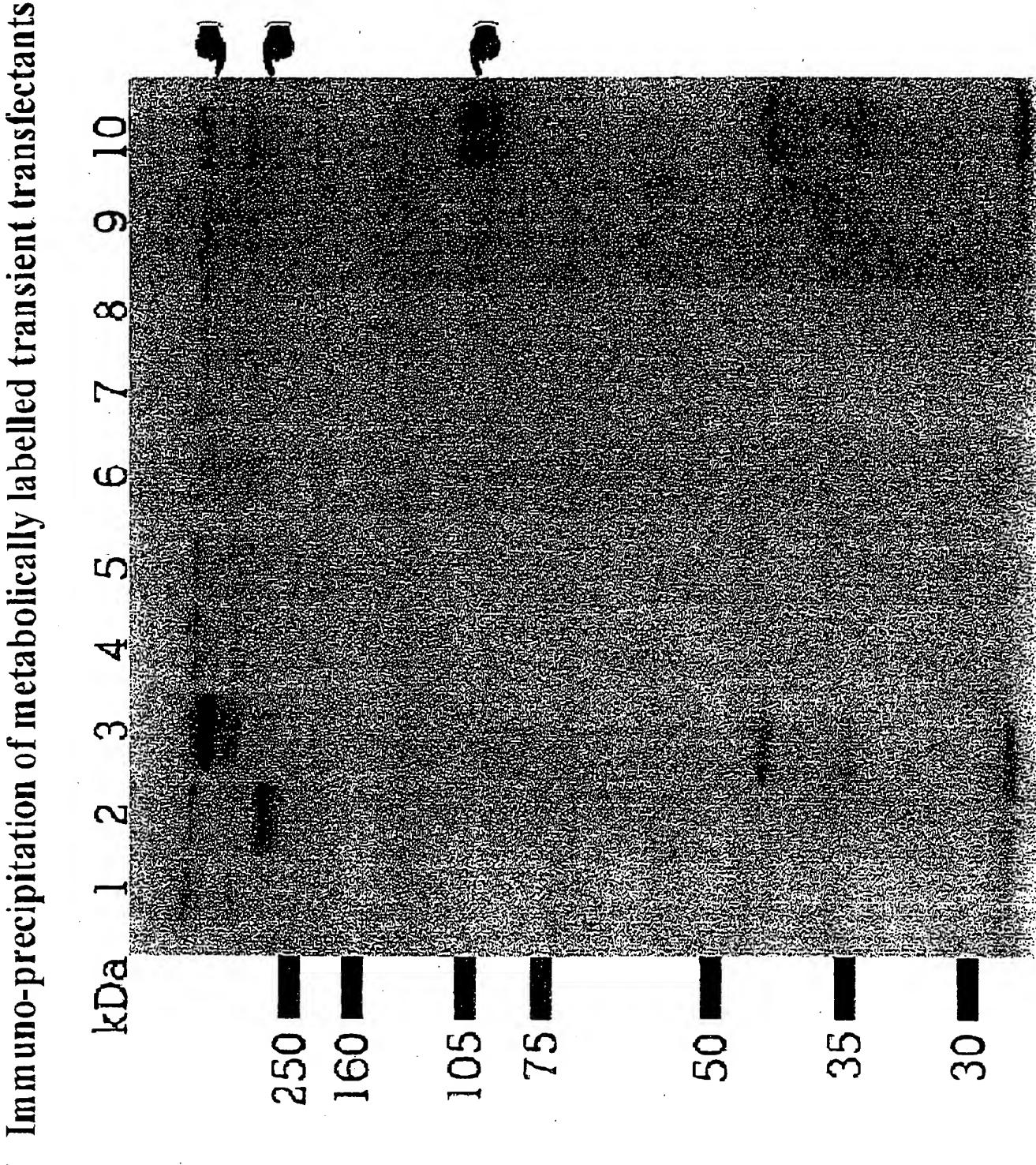


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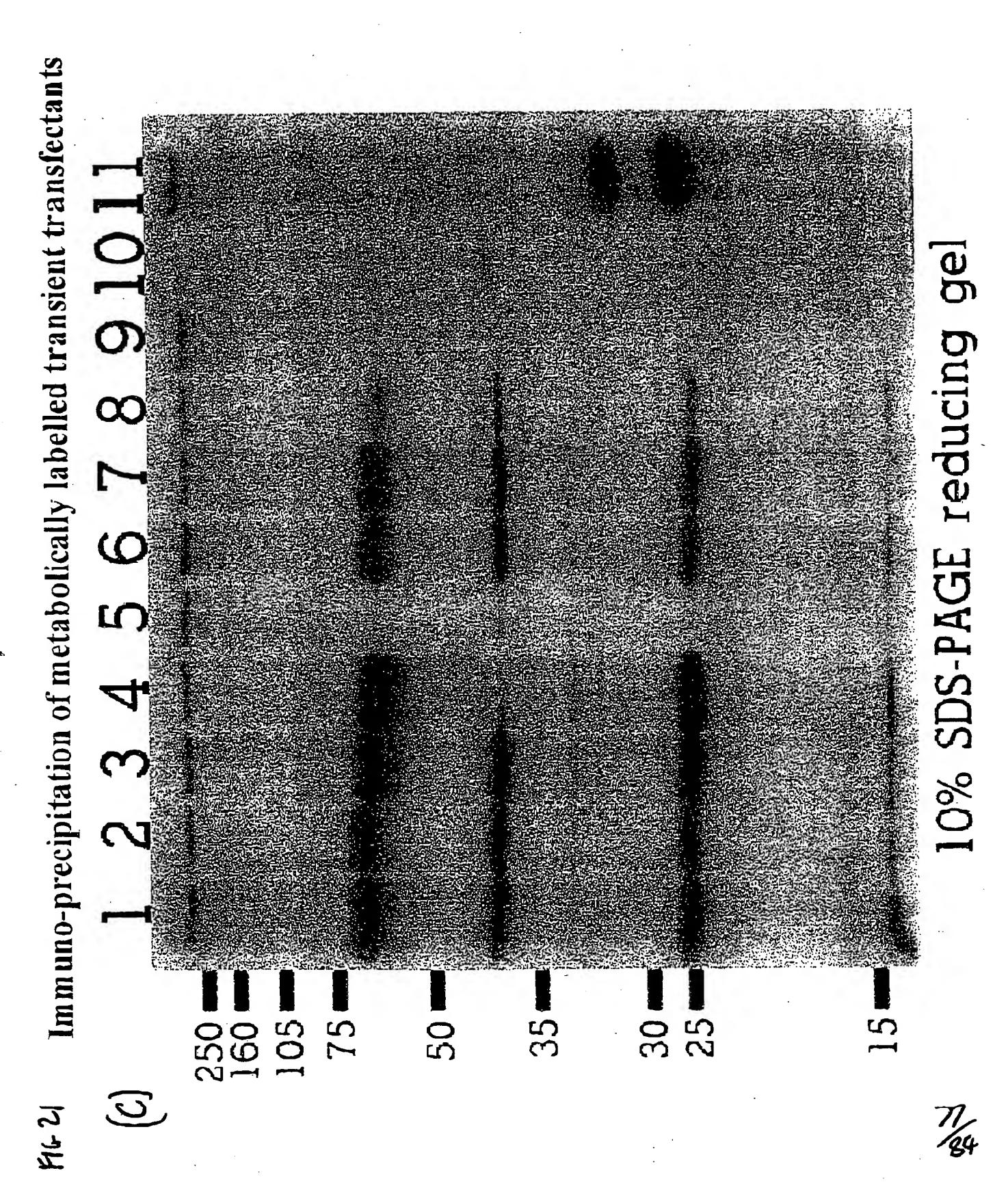




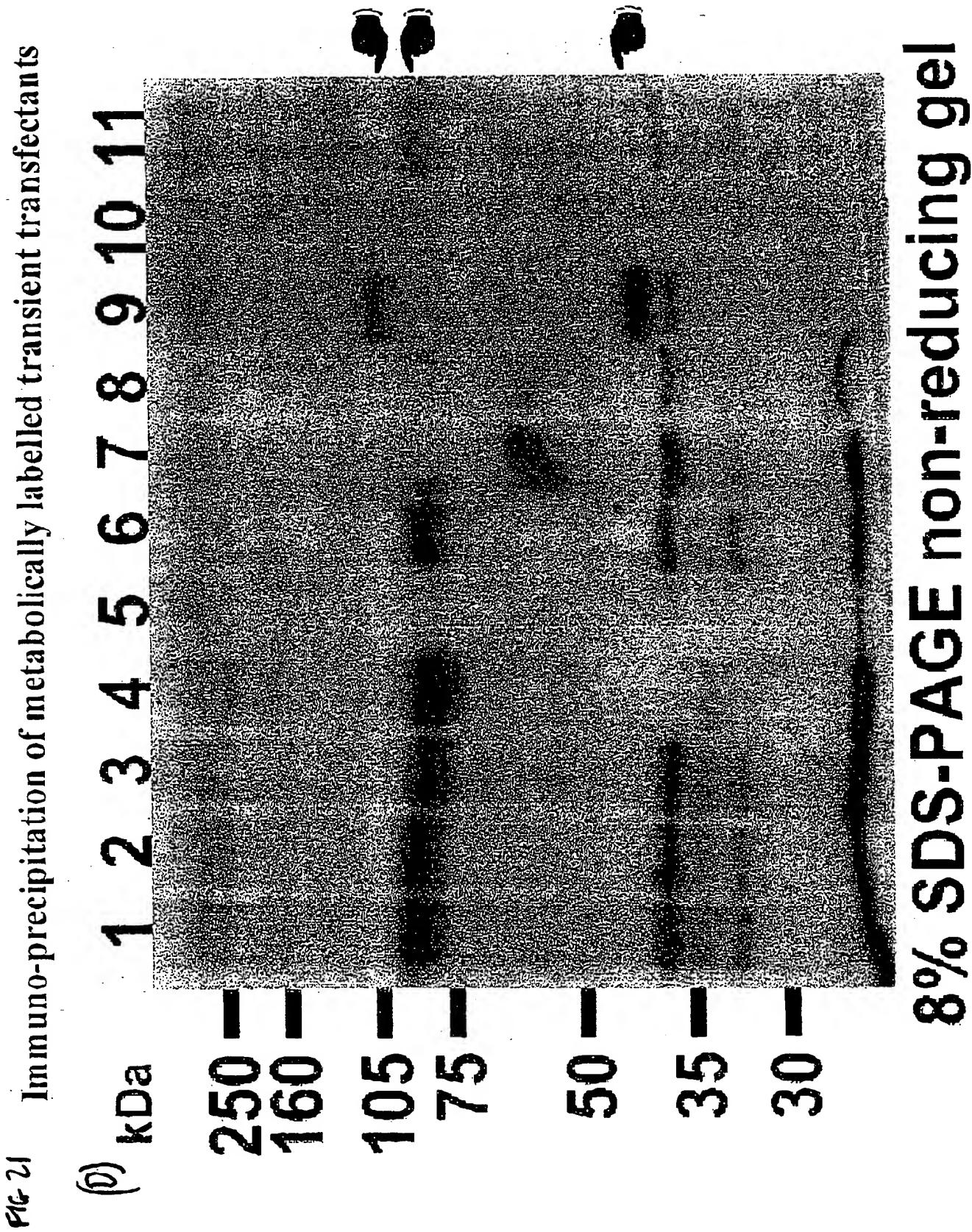


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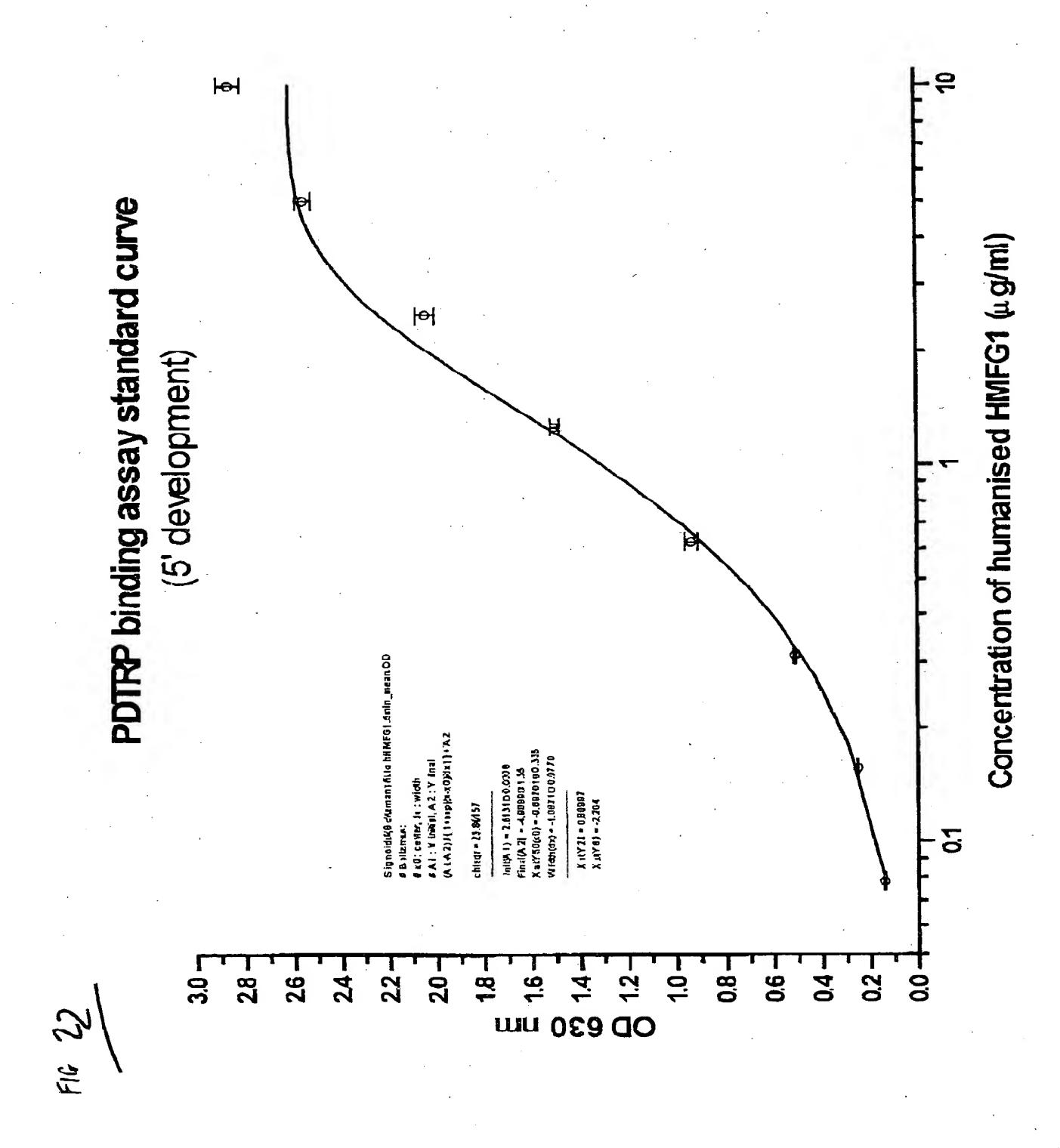














Corrected bovine DNase I standard curves

at various time points

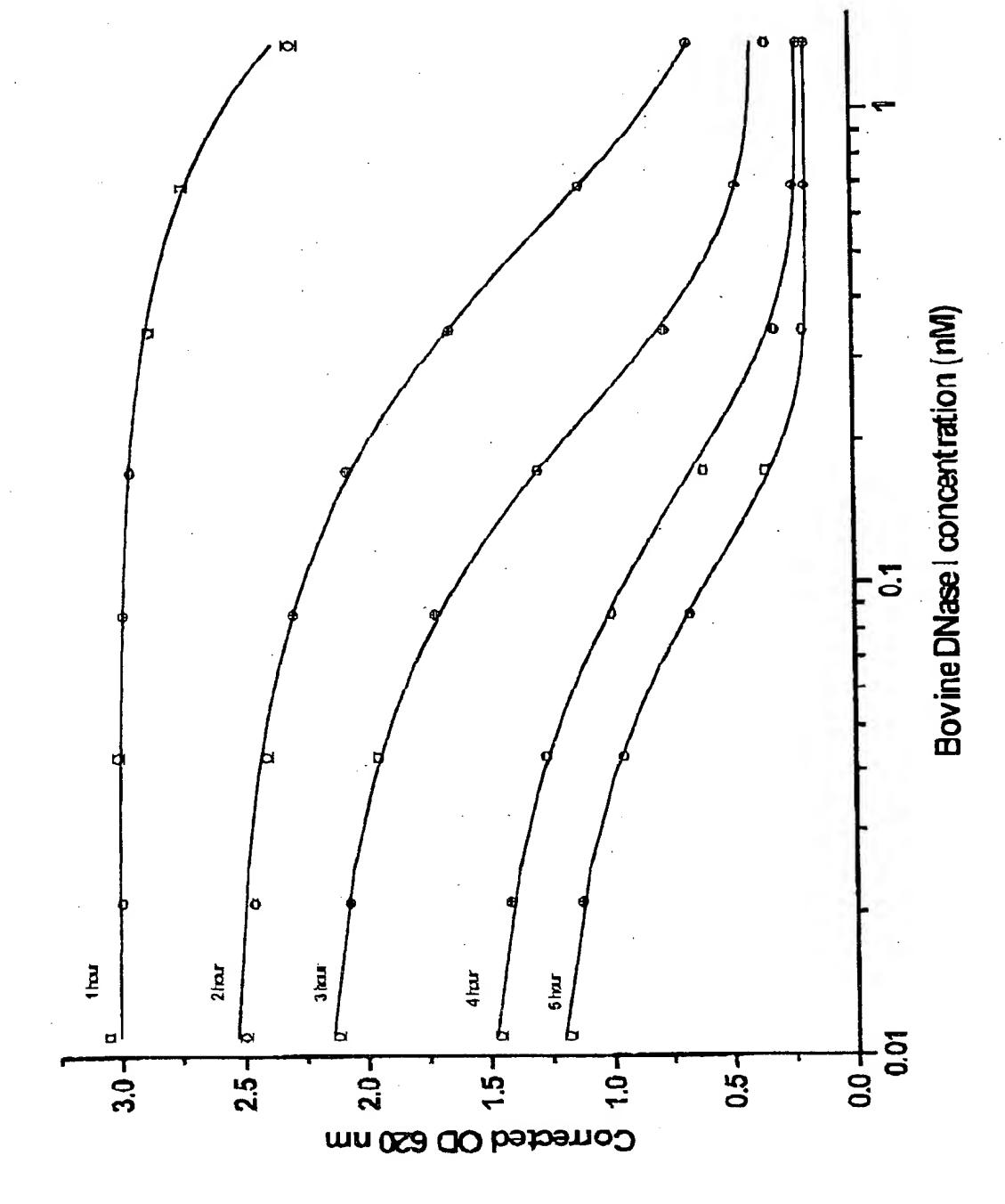
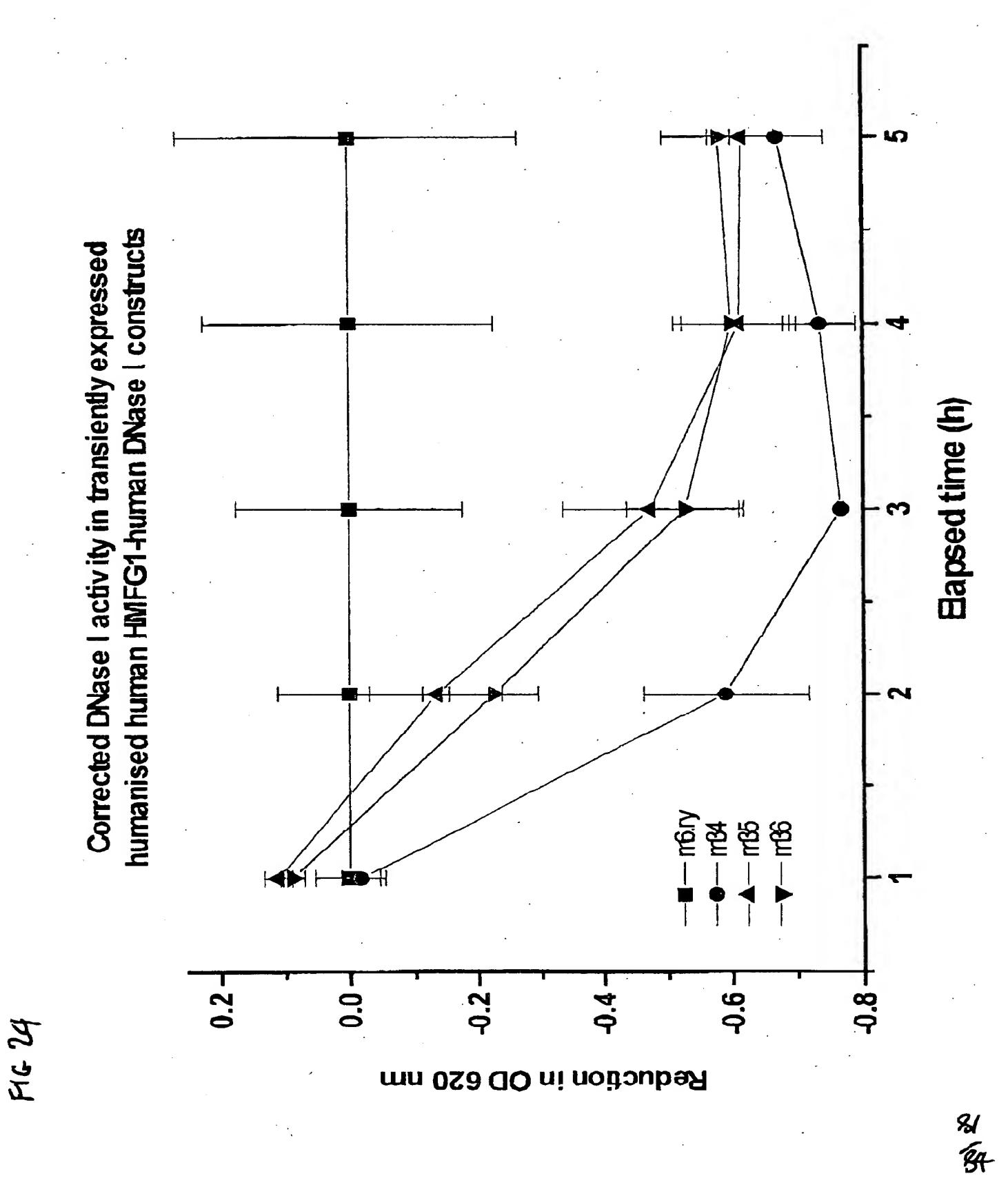


FIG 23

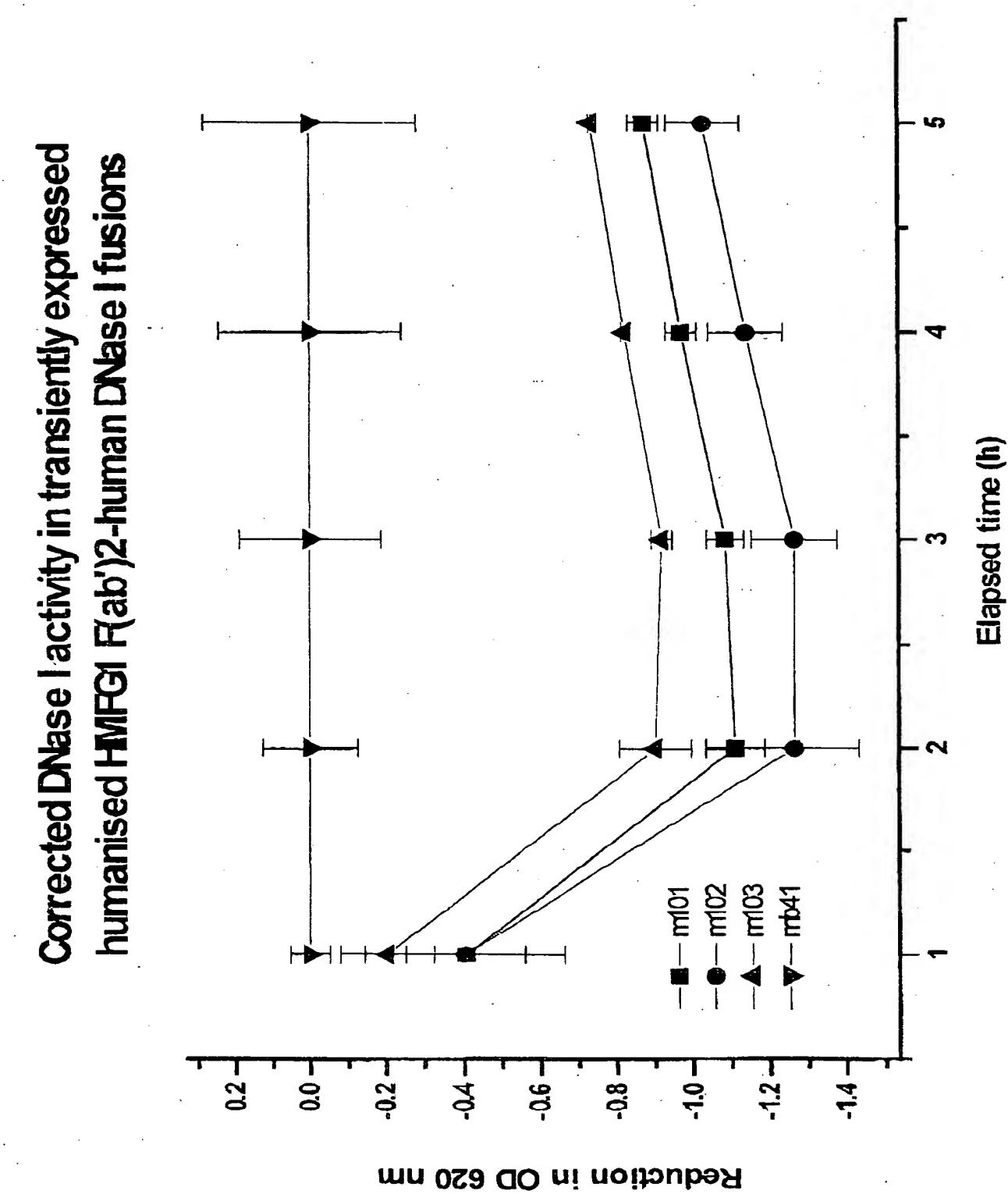




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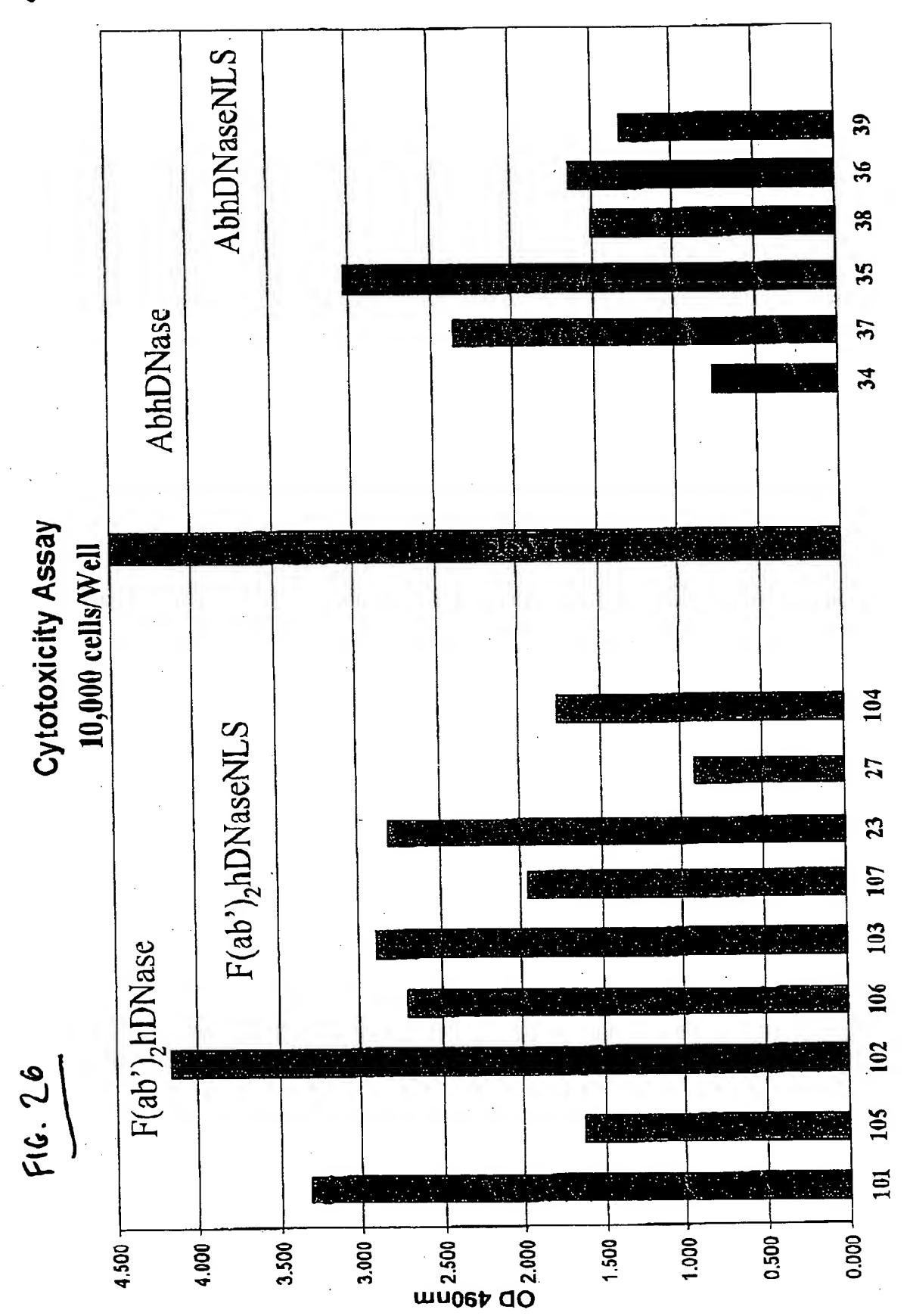
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To-THE PATENT OFFICE





0.097 μg/ml of each construct





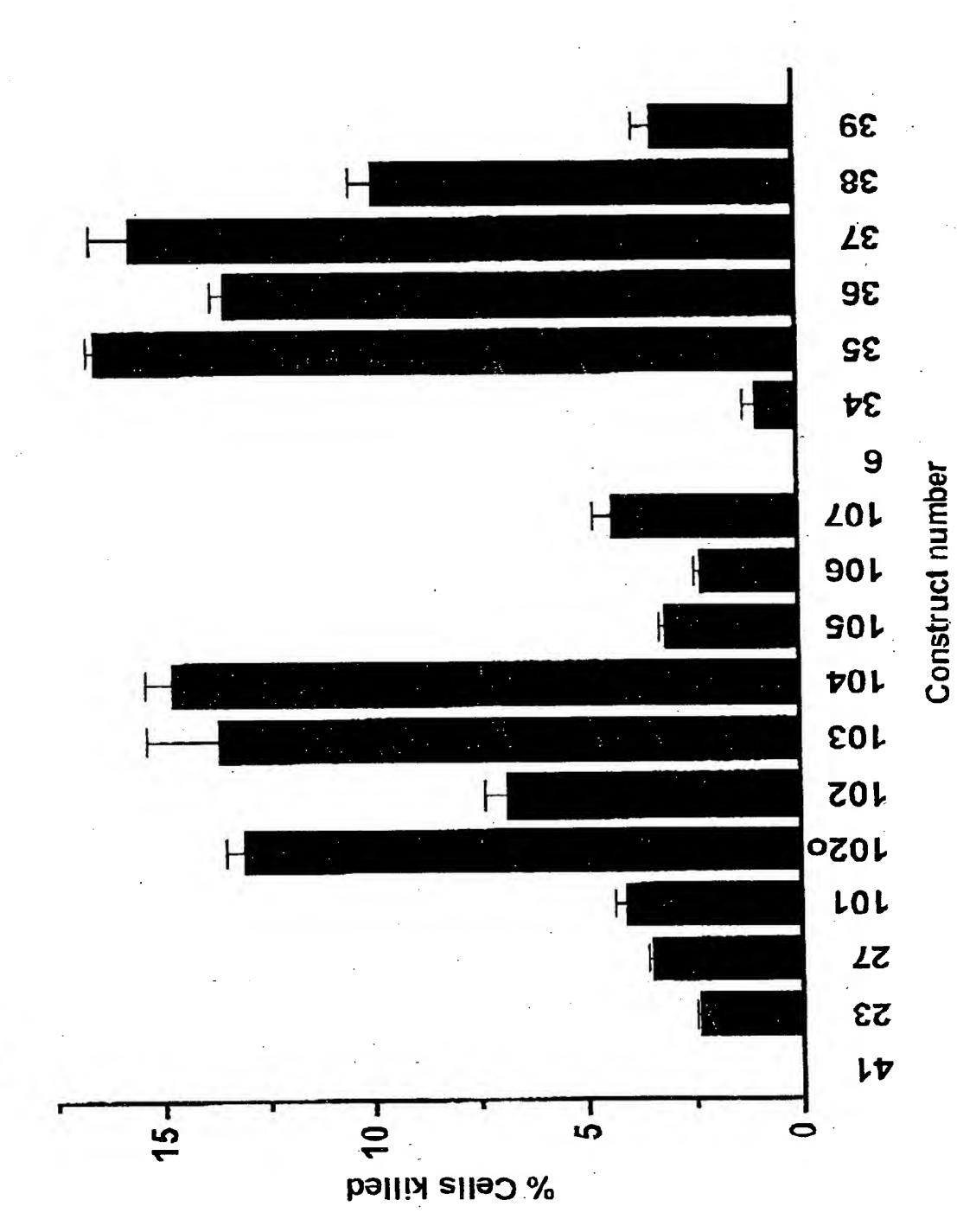


FIG 27

84

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